# STUDIES ON THE PLANT CELL. — V.

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### SECTION IV. CELL UNIONS AND NUCLEAR FUSIONS IN PLANTS.

THE forms of cell unions and nuclear fusions in plants fall into two groups : (1) those which obviously have no sexual significance attached to them, and (2) those which are evidently sexual acts. But apart from these simple divisions there are some very interesting conditions in which it is far from easy to determine whether certain events have a sexual significance either physiologically or phylogenetically. The real test of such problems should lie in the evolutionary history of the processes involved, for every sexual condition in plants has probably developed in obedience to the same physiological demands and in an essentially similar manner. However, we cannot apply the evolutionary test in many cases where we have little evidence of the developmental history of the group and such forms must rest for the present as unsolved problems. We shall treat them in special connections later in the paper.

The material of this section will be presented under the following heads : —

1. Protoplasmic connections between cells (plasmodesmen).
2. Sexual cell unions and nuclear fusions.
3. Asexual cell unions and nuclear fusions.

## 1. Protoplasmic Connections between Cells (Plasmodesmen).

It has been known for a great many years that the walls between the cells in some plant tissues and more especially between the cells of filaments in certain thallophytes were crossed by delicate strands of protoplasm so that contiguous protoplasts were not entirely separated from one another. This fact

offers at once many interesting possibilities of explaining the close association of many cells and tissues, not alone in delicate dynamic interrelations but even in the exchange and distribution of food material and other products of metabolism. It makes possible the conception of the plant body as a finely adjusted community of protoplasts intimately and sensitively related to a great degree in all parts, a view very different from the old idea of a cell republic. As might be expected, these speculative possibilities were conceived and expressed by such leaders as Hofmeister, Nägeli, Sachs, and Strasburger long before the detailed study of protoplasmic connections gave the mass of evidence upon which have been based the more elaborate conceptions of recent years.

The most obvious protoplasmic connections between cells may be found in the thallophytes where as in the Rhodophyceæ, Volvox, and in certain fungi, the cells in younger structures may be observed under comparatively low magnification to be united by strands of protoplasm so broad as to quite exclude them from the category of fibrillæ. Some of these structures are so conspicuous that it is surprising that more was not made of them by early writers and that they have not been more extensively investigated recently. The greater part of the papers have been on the very difficult phase of the subject, the structure of pores and pits in the tissues of higher plants. The literature treating of protoplasmic connections is too extensive to be given detailed treatment in the compass of this paper. The best review of the subject is that of Strasburger (: 01), supplemented by the more recent paper of Kienitz-Gerloff (: 02).

The earlier papers on the protoplasmic connections in higher plants, following the establishment of perforations of sieve-plates by Sachs and Hanstein, appeared during the years just preceding and following 1880. Thus Tangl ('79–'81) described very clearly the communications between the endosperm cells of *Strychnos nux vomica* and Phœnix (see Fig. 16, a). Tangl noted the resemblance of the complex of connecting threads to the arrangement of spindle fibers associated with the simultaneous division of the protoplasm in the endosperm but was cautious in assuming a relationship, suggesting that the resemblance might be superficial.

Strasburger ('82, p. 246) discussed the permeability of cell walls and Gardiner ('88) gave a general treatment of the subject without, however, any figures to illustrate his conclusions. Gardiner discovered for a large number of forms in a wide variety of families that the pit membranes were frequently pierced by protoplasmic fibrils and that in some cases the fibrils traversed the entire thickness of the cell wall. A more detailed study with better methods, supplementing his former work and accompanied by figures, was published by Gardiner, in 1898, this paper forming an important contribution to the subject. Gardiner (:00) announced himself strongly in favor of the view that the protoplasmic connections between cells were derived from spindle fibers of nuclear figures concerned with each cell division, a possibility which had been suggested by previous writers (Tangl, '79–'81 ; Russow, '83).

Kienitz-Gerloff ('91) gave an excellent account of the protoplasmic connections in a number of forms, some of them pteridophytes, but especially for *Viscum album*, and followed the history of the wall formation, showing that the spindle fibers disappeared completely before the development of the connecting strands of protoplasm. Kuhla (:00) followed Kienitz-Gerloff with more extended studies on the same form, *Viscum album*, tracing the protoplasmic fibrils between the cells in all the chief tissues and establishing the protoplasmic connections throughout the individual to an extent that was not known before. Hill (:01) described the structure of the sieve-tubes of Pinus, dealing especially with the formation of callus and the conversion of the connecting threads of protoplasm into strings of slime. An excellent review is also given of the work of Russow and others, particularly upon sieve-tubes. Kohl ('97) describes clearly protoplasmic connections between the cells of moss leaves.

A classification of protoplasmic connections was suggested by Kohl (:00) who distinguished between the solitary state when each fibril pierces the cell wall independently of its neighbors (Fig. 16, a and b) and a grouped condition when a number of fibers arise close together at the bottom of a pit and pierce the pit-membrane or middle lamella in a spindle-shaped arrangement,

reminding one of the central spindle of a mitotic figure (Fig. 16, c). In general the two types of protoplasmic connections are not found together in the same cell or tissue.

A new point of view was introduced into the discussion by the very important paper of Strasburger, in 1901. He considered the protoplasmic connections as sufficiently clearly differentiated structures to rank as organs of the cell and proposed for them the name plasmodesmen. Strasburger in agreement
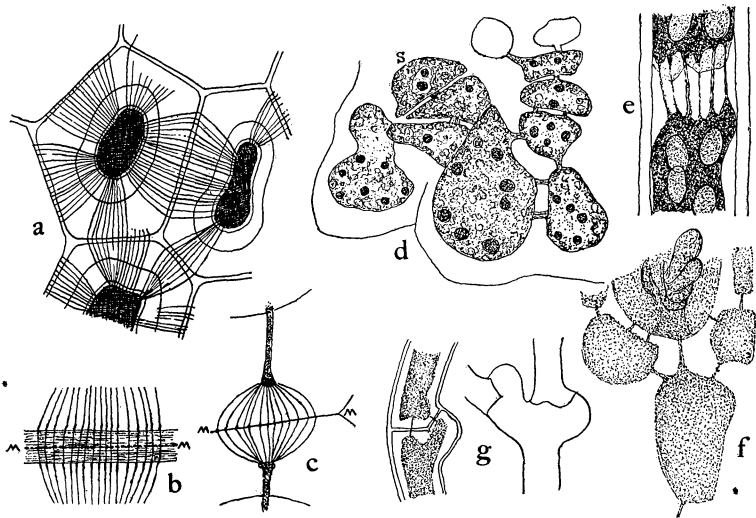


FIG. 16. — Protoplasmic connections between cells of plants. *a*, endosperm cells of *Strychnos nux vomica* ; *b*, details of the solitary fibers in the same form, (*m*) middle lamella ; *c*, grouped fibers at bottom of pit in endosperm of Phytelephas and crossing the pit membrane in a spindle-shaped figure ; *d*, cell connections around the sporophytic portion of a developing cystocarp of Champia, (*s*) sporophytic elements ; *e*, fibers between cells of Cladophora ; *f*, cell connections around the ascogenous elements in Laboulbenia ; *g*, clamp connections in Pleurotus (*a*, after Tangl, '79–'81 ; *b* and *c*, Kohl, :00 ; *d*, Davis, '96 b ; *e*, Kohl, : 02 ; *f*, Thaxter, '96 ; *g*, Meyer, : 02).

with Kienitz-Gerloff opposed the view that the plasmodesmen were in any way derived from or related to the spindle fibers associated with the formation of cell plates. He believed them to be developments of the outer plasma membrane as he likewise considers the cilia in certain zoöspores (see account of zoöspore and sperm in Section III, *Amer. Nat.* vol. 38, pp. 571, 576, 1904). Strasburger also holds that pores are formed in the cell walls by the fermative activities around plasmodesmen. A

recent short paper by Michniewicz (:04) describes clearly the plasmodesmen in Lupinus, especially in their relation to masses of intercellular protoplasm which are discussed at the end of this portion of the section.

It is not clear whether all protoplasmic connections may be considered in the same class, as Strasburger would have us believe, or whether there may not be some confusion between the broader cell connections which are especially conspicuous in the thallophytes and certain tissues (sieve-tubes, laticiferous vessels), and the delicate protoplasmic fibrils (plasmodesmen) so general throughout all tissues of higher plants. As is well known, the cells in actively growing regions of the red algæ are connected by broad strands of protoplasm that are obviously left by the cleavage furrow which constricts the protoplasm of daughter cells but does not entirely separate them. These openings may become partially blocked in older portions of the plant by the deposition of material so that the connections are finally fibrillar but they frequently remain open for long periods, particularly in regions where the nutritive processes are active as during the development of cystocarps. At this time new fusions may be developed between neighboring cells (auxiliary cells) so that they become connected in an elaborate network around the cells or filaments (sporophytic) that develop the carpospores (Fig. 16, d). The Phæophyceæ also furnish frequent illustrations of connecting fibrils especially in the Fucales and Laminariales where the cells of internal filaments are sometimes connected by conspicuous strands. Certain elongated filaments which traverse the central region of the larger brown algæ show a complicated group of fibrils that strikingly resembles the protoplasmic connections piercing the sieve-plates of higher plants. Broad protoplasmic connections are conspicuous between the cells of some of the filamentous Cyanophyceæ (Stigonema, Tolypothrix) and in the Chlorophyceæ have been reported for some species of Cladophora (Kohl, :02 ; Fig. 16, e) and for Chætopeltis, one of the Mycoideæ. They do not seem to be present in the Conjugales as was at first reported by Kohl ('91) whose cells show a great degree of physiological independence. In Volvox, studied by Meyer ('96), each cell of the

sphere is connected with its neighbors generally by six strands of protoplasm, only a few of which could possibly be left by the successive cell divisions. The majority must have developed as outgrowths from the plasma membrane of the cell.

Numerous instances of cytoplasmic connections among the fungi have been reported by many authors. A general review of the subject is presented by Kienitz-Gerloff (:02) and in a lengthy paper of Meyer (:02). The protoplasmic connections fall into two groups: (1) those that remain in the center of the wall after a cell division, and (2) the lateral unions and clamp connections which are developed entirely independently of cell division. Connections of the first type, *i. e.*, those between daughter cells, appear to be very general in the Ascomycetes and Basidiomycetes and are essentially similar to the strands between cells of the Rhodophyceæ. They are especially well illustrated in members of the Laboulbeniaceæ (Thaxter, '96 ; see Fig. 16, f ). In the second group are the clamp connections (Fig. 16, g), characteristic structures of the tissues of fleshy forms of the Basidiomycetes, and the lateral unions between cells of closely entangled hyphæ which are well known in a number of forms and have been followed in cultures from germinating spores. It is probable that the fusions between sporidia in the smuts are also of this class, although De Bary and others have attached sexual significance to the phenomenon (especially as illustrated by Tilletia). Harper ('99a) has studied the fusions of the conidia of Ustilago and finds that they concern the cytoplasm alone. However, Federley (:03–:04) has reported a nuclear fusion in one species (*Ustilago tragopogonis pratensis* Pers.) but states that others agree with Harper's account. Extensive experiments of Brefeld have shown that the fusions of sporidia depend largely upon the character of the nutrient media and are less likely to occur when the conditions are favorable. He considers the fusions as purely vegetative processes comparable to the unions of germ tubes of spores (*e. g.*, Nectria, Sclerotinia, Rhyparomyces, etc.) into a common mycelium and to the connections between hyphæ of Basidiomycetes. Recent studies of Blackman (:04 a) indicate also that sexual processes should not be expected at this period in the life his-

tory of smut or rust. One of the best discussions of cell fusions in the fungi is that in Harper's paper ('99 a), noted above.

Although most of the protoplasmic connections in higher plants are of the fibrillar character there are some notable illustrations of broad openings between cells, even more conspicuous than those in the red algæ. Such may be found in the pores of sieve-plates traversed in their early stages by strands of protoplasm that later disappear, and even better illustrations are the unions between cells composing laticiferous vessels. But the most interesting conditions are those associated with the nutrition of the eggs of certain cycads. Goroschankin ('83) first noted for the cycads pores or canals in the egg-wall of Ceratozamia and described communications between the protoplasm of the enveloping cells of the jacket and the egg. The subject is closely associated with the explanation of the proteid vacuoles in the eggs of gymnosperms which Arnoldi believed to be nuclei that had migrated from the surrounding cells. The conclusions of Arnoldi have not been sustained (see Sec. III, *Amer. Nat.*, vol. 38, pp. 591, 592, 1904) but the presence of pores in the egg-wall of gymnosperms is likely to prove very general with further investigation. A recent paper by Miss Isabel Smith (:04) gives an account of haustoria-like processes from the egg of Zamia which pass through the pores of the egg-wall into the cells of the jacket, where they are in direct contact with its protoplasm. These pseudopodia-like processes of the egg apparently absorb material from the cells of the jacket as is indicated by the character of their staining and the streaming movement towards them of the protoplasm in the jacket cells. The relation of the plasma membrane of the processes from the egg to that of the jacket cells is not clear but probably they are merely in contact and not in open communication. The ovules of cycads seem to offer an especially favorable subject for the study of pore formation and the intimacy of protoplasmic connections between cells.

It seems very clear that the cytoplasmic connections in the Rhodophyceæ, Volvox, fungi, and between the egg and jacket cells of cycads involve very much more substance than is generally present in the delicate fibrillæ of higher plants. Meyer

(: 02, pp. 167, 168) seems justified in emphasizing their resemblance to pseudopodia rather than to any other structure of the cell. If they should finally be connected by intergradations with the exceedingly fine plasmodesmen of Strasburger, there would stand at one end of the series structures so thick as to be composed of a plasma membrane containing much cytoplasm in the interior and behaving like haustoria or pseudopodia and at the other end delicate fibrillæ. Viewing the problem of their relationships from the lower plants upwards, it is very difficult, if not impossible to follow Strasburger's theory that all cytoplasmic connections (plasmodesmen) are related to developments from the plasma membrane similar to cilia. They seem to be more of the nature of processes put out from the cytoplasm and when necessary penetrating cellulose walls probably in response to chemotactic stimuli since they are most conspicuous when metabolic activities are obviously important (*e. g.*, nourishment of the egg in gymnosperms and sporophytic generation of the red algæ).

In method of development we have seen that protoplasmic connections fall into two classes : (1) those that represent the incomplete separation of daughter cells, and (2) those that result from the coming together or fusion of protoplasmic outgrowths. The types of the first group are always in the beginning open communications which later may become largely or wholly closed ; types of the second group may result in broad cytoplasmic fusions (*e. g.*, many fungi) but there is evidence that in many cases, especially among the higher plants, the two processes only come in contact so that the plasma membranes are applied to one another but do not actually unite. It does not seem probable that the two methods of development or the presence or absence of intimate protoplasmic union indicate a different kind of structure. They are more likely to be only varied responses to the demands for a more or less close association of neighboring cells. Broad communications are especially characteristic of regions where there is evidently an extensive demand for the nourishment of a cell or tissue, as in the eggs of the cycads or the cystocarp of the red algæ.

The functions of protoplasmic connections are probably vari-

ous. It is evident that they bind the whole plant body into a cell complex capable of very delicate interrelations. It is natural that physiologists, Pfeffer and others, should associate the structures with the phenomena of irritability as the paths over which stimuli may be transmitted from cell to cell and tissue to tissue. Several writers have reported their presence in unusual numbers in irritable structures of plants. The subject is discussed in great detail by Strasburger (:01, p. 533).

Besides conducting stimuli, there is much evidence that material may be transferred in solid or semifluid form by the protoplasmic connections from cell to cell and that in some instances there is actually a movement or flow of protoplasm. It is even known that nuclei may pass from cell to cell through pores in the wall, especially after some shock, as in the neighborhood of wounds (Miehe, :01), or when temperature is suddenly raised (Schrammen, :02). This literature and other references are discussed by Koenicke (:01 ; :04). A flow of protoplasm between neighboring cells of hyphæ has been reported by Reinhardt ('92) and Charlotte Ternetz (:00). That nuclei may pass through very small space is shown in the development of spores in the Basidiomycetes and in the growth of haustoria from the cells of hyphæ (Smith, :00). There are many forms known, especially among the thallophytes, where the communications between cells are so broad as to admit of a very free circulation of their contents. Such conditions are especially well illustrated in tissues around the developing cystocarps of the Rhodophyceæ and the ascocarp of the Ascomycetes, both structures apparently sporophytic in charater and dependent to a great degree upon the gametophyte as a host. It is believed that the vitality of protoplasm in sieve tubes, whose nuclei have degenerated and disappeared, is maintained through protoplasmic connections with neighboring cells and especially the companion cells, when present. Of course where an actual circulation of protoplasm is established between cells or tissues there is made possible a distribution of the products of metabolism in solid form that is very different from the usual diffusion in tissues through cell walls and plasma membranes.

It seems probable that there are really two forms of proto-plasmic connections between cells in plants : first, those so intimate that the plasma membranes are pierced and become continuous openings inclosing a strand of granular cytoplasm within ; and second, those in which the plasma membranes are merely applied to one another without open communication. The second form comprises the most delicate connecting fibrillæ, structures so fine that their minute structure is not understood and we do not know how intimate may be the application of the fibrillæ to one another or to the surface of the cells. These are the typical plasmodesmen of Strasburger which he considers as organs of the plasma membrane, kinoplasmic in character, and compares to cilia. The broad connections of the first group have exactly the structure that would be expected of fused pseudopodia, as Meyer pointed out. Whether the two types insensibly grade into one another or whether each is a develop-ment by itself is a problem of considerable interest, for if the former possibility prove true, Strasburger's conception and clas-sification of plasmodesmen as organs of the cell will hardly seem justified.

When protoplasmic connections become so broad that cyto-plasm flows or surges from one cell to another, an actual transfer of nuclei sometimes takes place. Such conditions may illustrate simply one extreme of the series of protoplasmic connections that we have just discussed, but many of them introduce some complexities, mainly through a certain resemblance to sexual processes, so that they should be treated apart from general protoplasmic connections. Some of them will be described later under the head of " Asexual Cell Unions and Nuclear Fusions."

Closely associated with protoplasmic connections is the inter-esting subject of intercellular protoplasm which is receiving some attention at present. The last papers are by Kny (:04) and Michniewicz (: 04) who are studying conditions in the seed, especially of Lupinus. By various reactions and physiological studies, Kny has established an apparent identity of nature between an intercellular substance, sometimes with starch inclusions, and the cytoplasm of the neighboring cells. He considers this substance to be intercellular protoplasm, that is,

protoplasm outside of the cell walls, but connected with the cytoplasm within through fibrillæ.    The intercellular proto-plasm is thus conceived in organic connection with nucleated cells and from the studies of Townsend ('97) we know that non-nucleated protoplasm may live so long as it is united with nucleated, even though it be by very delicate fibrillæ.    Michnie-wicz (: 04) confirms Kny's conclusions for Lupinus and gives a very clear account of the fibrillæ which connect the masses of intercellular protoplasm with neighboring protoplasts.    These studies make clearer a number of observations of several inves-tigators (Sauvageau, Buscalioni, Schenk, Magnin, Strasburger, and others) who have noted similar conditions in the tissues of higher plants which are being investigated in detail by Kny. Some of the lower unicellular forms likewise exhibit an extra-cellular surrounding film or envelope, which may also be of a protoplasmic nature and consequently in the same position in relation to the protoplast as intercellular protoplasm.    Thus it has been known for many years that the cells of the Peridinales, diatoms, and desmids possessed extracellular material, which some authors have considered in the nature of slimy excretions but others — Schutt ('99; : 00a; : 00b), Hauptfleisch ('88 ; '95), Müller ('98–'99) — have regarded as protoplasmic in character. Since the cell walls in these forms are known to possess pores, such extracellular substance must be in close association with the cytoplasm of the cell and it is not at all difficult to conceive of it as a part of the protoplasm.    Some of the peculiar creep-ing movements of the diatoms and desmids are perhaps expli-cable upon these facts.

## 2.   Sexual Cell Unions and Nuclear Fusions.

The test of a sexual act must lie with the history of the ele-ments which fuse.    If these are shown by their morphology and developmental history to be sexual cells or gametes then their fusion becomes a sexual process.    There are cell and even nuclear fusions which have the physiological appearances of sexual acts but cannot be so considered because the elements concerned have  plainly no relation to sexual cells, which are

developed at other periods of the life history, or to the primitive conditions always found with the origin of sex.   These exceptional processes will be collected and described under the heading "Asexual Cell Unions and Nuclear Fusions," following this portion of the paper.

The union of gametes is generally termed fertilization.   The evolution of the sexual process always tends towards a differentiation of the two sexual cells, one becoming more richly stored with food material and containing more protoplasm than the other.   This latter gamete is always considered the female and is said to be fertilized when the male gamete, either as a motile sperm or reduced simply to a sperm nucleus generally with some accompanying protoplasm, fuses with it.   The most evident morphological feature of fertilization is the close union of the gamete nuclei so that the chromosomes of both enter into the mitotic figure with which the new generation begins.

We shall not discuss the various forms of gametes nor their habits in different types of sexual reproduction.   They have been described in two articles by the author on the origin and evolution of sex in plants (Davis, :01 ; :03).   A detailed account of the sexual reproduction of well known types throughout the plant kingdom has been recently published by Mottier (:04b) under the title "Fecundation in Plants" a term which he prefers to fertilization.   This paper gives in English the most extensive summary of our knowledge of the subject up to the date 1902 and will be read with especial interest as the most available expression in English of Strasburger's general views on the significance of the events connected with sexual reproduction.

A recent paper of Guérin (:04) is confined to an account of fertilization in the phanerogams which are treated in considerable detail.   His discussion of double fertilization and parthenogenesis is of especial interest and will be taken up later.

Our purpose is to divest from the events of sexual cell unions and nuclear fusions all secondary and unessential processes and to outline, as are now understood, the fundamental phenomena. And to make the subject more plain we shall try to compare in their essentials the events of fertilization in plants with those

in animals.   Probably the most important feature of fertilization is the close union of the gamete nuclei through which the chromosomes of both enter into the first mitotic figure of the new generation.   It involves the organization of the first cleavage spindle, which inaugurates the new generation, and the history of the paternal and maternal chromosomes of the gametes at this time when the number becomes doubled.

Several zoölogical papers have developed in the past few years some very important conclusions concerning the individuality of the paternal and maternal chromosomes, as maintained during the fusion of the gamete nuclei and in the formation of the first cleavage spindle.   It has been generally believed for some time — see general review in Wilson (: oo, p. 204) — that the fusion of gamete nuclei did not involve a coalescence of the chromosomes but that both paternal and maternal chromosomes maintained complete independence of one another and that all entered into the first cleavage spindle as structures quite as distinct as when formed during spermatogenesis and oögenesis.   Häcker and Rückert have shown for Cyclops that the gamete nuclei divide side by side in the first mitosis following fertilization, and Häcker followed these double nuclei as far as the 16-celled stage when they were still distinct from one another.   A few notable investigations of recent years have identified chromosomes accurately as maternal and paternal not only in the first cleavage spindle but through certain succeeding mitoses and finally at the period of gametogenesis when sperm and egg were again formed.   The above principles have been established chiefly through a series of papers of Montgomery, the chief being a lengthy investigation of 1901, and contributions of Sutton (: o2 ; : o3) and Moenkhaus (: o4).   They have given us clear evidence that the chromosomes not only maintain their complete individuality throughout successive generations but are distributed with gametogenesis and fertilization in various possible combinations that can be expressed by mathematical formulæ furnishing the basis for certain ratios that approximate the teachings of Mendel's law.   We shall have occasion to refer to these in Section V when the subjects of gametogenesis, reduction of chromosomes, and hybridization will be discussed.

The same principles have been established in plants by recent investigations, some ot which deal with oögenesis and spermato-genesis and will be specially treated in the Section V while others treat of the behavior of the chromosomes when the gamete nuclei fuse and the sporophyte generation begins its development. The latter conditions concern the present discussion.

The history of the chromosomes in plants at the time when the gamete nuclei fuse (fertilization) is most accurately known for the pine. The last paper upon this type (Ferguson, :04) is very complete. Miss Ferguson gives a beautiful series of figures, some of which we have reproduced. The sperm nucleus comes in contact with the egg nucleus and sinks into the latter so that it lies in a depression, but as noted by Blackman ('98), it does not penetrate the membrane of the egg nucleus (Fig. 17, a). Both gamete nuclei thus lie side by side occupying approxi-mately the same space formerly filled by the female. Each shortly gives evidence of preparation for the mitosis following fertilization (first cleavage spindle). The chromatin of the egg nucleus collects in a spirem, very close to the sperm, occupying a relatively small portion of this large female nucleus (Fig. 17, b). The chromatin of the sperm nucleus also takes position as a spirem on the side nearest its companion chromatin of the oppo-site sex. The remaining space of each nucleus is filled with a granular reticulum of a linin nature. At this time the amount of linin is extraordinarily large in proportion to the chromatin, suggesting that some of the latter substance has become changed to the former. Soon, delicate fibrillæ appear around the two spirems growing outward in various directions and finally cross-ing from one nucleus to the other. At the same time the two nuclear membranes become less distinct and shortly disappear. Thus the maternal and paternal spirems come to lie in a com-mon area filled with delicate fibrillæ which run out to the gran-ular cytoplasm that lay around the two gamete nuclei (Fig. 17, c). It should be especially noted that at no time in this history has there been a resting nucleus including both maternal and pater-nal chromosomes within a common nuclear membrane. The fusion of the gamete nuclei has only come with the actual formation of the first cleavage spindle.

The fibrillæ organize a multipolar spindle which is very variable in form, sometimes with broad poles of a multipolar diarch (Fig. 17, d) and at other times almost as pointed as in a typical bipolar spindle (Fig. 17, e).   There are, of course, no centrosomes and the entire spindle in essentially of intranuclear origin. The history of its development recalls Miss Williams' account of the spindle in the pollen mother-cell of Passiflora (Sec. III, *Amer. Nat.*, vol. 38, p. 738, 1904).   During spindle formation the spirems of the sperm and egg nuclei can be readily distinguished as was described by Blackman ('98) and Chamberlain
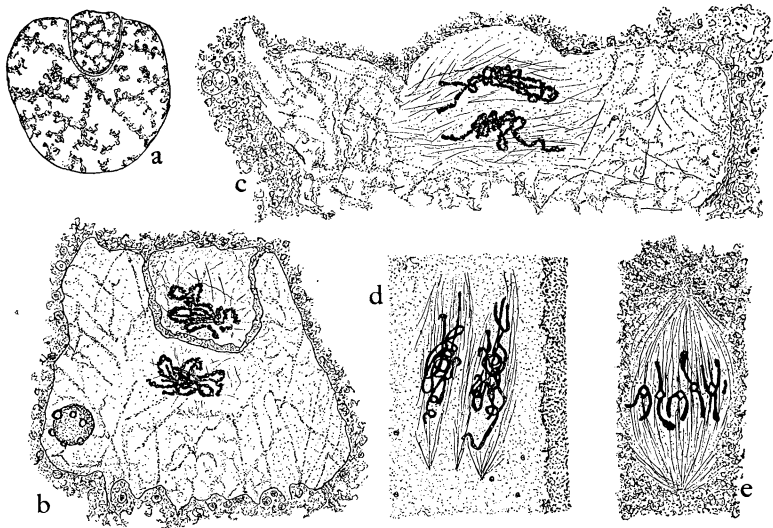


FIG. 17.— Fertilization in *Pinus strobus*.  *a*, conjugating gamete nuclei ;  *b*, the gamete nuclei still separated, with nuclear membranes distinct, the maternal and paternal chromatin in two spirems ;  *c*, the nuclear membranes have disappeared and the two spirems lie close together surrounded by the fibrillæ which will organize the first segmentation spindle ;  *d*, prophase of the first segmentation spindle, of the multipolar diarch type, paternal and maternal spirems still distinct ;  *e*, metaphase of first segmentation mitosis, maternal and paternal chromosomes now indistinguishable, beginning to split in the middle region (after Ferguson, :04).

('99), but after the two sets of chromosomes are formed (twelve of each) the latter are brought so closely together at metaphase of mitosis that the paternal and maternal cannot be separated. All of the chromosomes are exactly alike and there is nothing in the form or size to distinguish one from another as certain

zoölogists have been able to do in some favorable animal types (Montgomery, Sutton, Moenkhaus). The chromosomes divide longitudinally in the usual way, the halves being drawn apart from the points of attachment of the spindle fibers (Fig. 17, e). It is clear that each daughter nucleus receives a full set of 24 daughter chromosomes, 12 of paternal and 12 of maternal origin, and that there is about an equal amount of chromatin from each sex.

It should be especially noted that in the process of fertilization in the pine there is at no time present what is generally called a fusion nucleus, *i. e.*, a single nucleus whose membrane incloses all the material of both male and female gamete nuclei. Such fusion nuclei, as we shall see, have been reported many times in other groups of plants than the gymnosperms where in many cases, however, detailed studies are very difficult and can scarcely be said to have even approached our knowledge of the pine.

Studies of other botanists indicate that the gymnosperms generally will show essentially the same conditions as in the pine. Thus Woycicki ('99) distinguished in Larix two groups of chromatin which he regarded as paternal and maternal. And Murrill (: 00) states for Tsuga that the chromatin of sperm and egg remain separate, forming two spirems, and only after their segmentation into chromosomes are the two sets of structures brought together in the first cleavage spindle. Land (: 02) figured the sperm nucleus of Thuja imbedded in a depression of the egg nucleus. Miyake (: 03a) noted that the sperm nucleus of Picea became more or less imbedded in the egg nucleus while the nuclear membrane remained intact, and the same author (Miyake, : 03b), reports similar conditions in Abies. Robertson (: 04) figures the sperm nucleus of Torreya lying within a depression in the female and with a large amount of granular cytoplasm (kinoplasm) at the side. Coker (: 03) states that the partition between the gamete nuclei of Taxodium " does not entirely disappear until immediately before the first division " although the two structures are closely united for some time previously while they pass to the bottom of the egg.

Lawson, studying Sequoia (: 04a) reports gamete nuclei of

about equal size whose chromatin contents unite in a fusion
nucleus to form a common network in which male and female
elements cannot be distinguished. A similar condition obtains
in Cryptomeria, according to Lawson (:04b), where a fusion
nucleus is described in which paternal and maternal chromatin
are mingled together in a nucleus that passes through a short
period of rest before the development of the first cleavage
spindle. In view of the work on Pinus I think it may safely be
questioned whether in Sequoia and Cryptomeria the maternal
and paternal chromatin really does form a common network in
the resting fusion nucleus. The subject is one very difficult of
study and demands more stages than Lawson seems to have
followed.

Fertilization in the cycads is not as completely known as for
the conifers. Webber (:01) figures the sperm nucleus of
Zamia imbedded in the egg nucleus but quite distinct from it as
in the pine but the further history leading to the development
of the first segmentation spindle was not followed. On the
other hand Ikeno ('98b) described in Cycas the formation of a
cup-like depression in the egg nucleus to receive the sperm
nucleus which was said to enter and fuse completely with the
female and the same author (Ikeno, : 01) reports a complete
fusion of the gamete nuclei in Ginkgo and did not distinguish
the paternal and maternal chromosomes during the formation of
the first segmentation spindle. However it is probable that
more detailed studies among the cycads and in Ginkgo will
show a behavior of the sperm nucleus together with the pater-
nal and maternal chromatin essentially similar to that of the
conifers. All investigations among the cycads and in Ginkgo
agree that cytoplasmic structures of the sperm (blepharoplasts,
cilia, etc.) are left behind in the cytoplasm of the egg before the
gamete nuclei unite.

Our knowledge of the details of fertilization in the angio-
sperms is surprisingly meager. The only account of the chro-
matin is that of Mottier ('98 ; :04b, p. 176) for Lilium. He
describes and figures the two gamete nuclei as uniting with
their chromatin in the resting condition. The nuclear mem-
branes disappear at the surface of contact and the two nuclei

fuse into one.  The nucleoli unite and so thoroughly does the
paternal and maternal chromatin seem to be mixed in the resting
condition that the fertilized egg nucleus can scarcely be distin-
guished from the unfertilized.  There would seem to be then a
fusion nucleus in the lily with the chromatin in the resting
condition.  The figures and brief accounts of other botanists
indicate that similar conditions may be expected in other angio-
sperms.  But no one has followed the chromatin in the fusion
nucleus through its later history, during the organization of the
chromosomes preparatory to the first mitosis following fertiliza-
tion.  It would be very surprising if paternal and maternal
chromatin did not remain entirely independent of each other
as in the pine.  The detailed study of fertilization in the angio-
sperms presents a very attractive subject for investigation.

Some very interesting conditions of fertilization have been
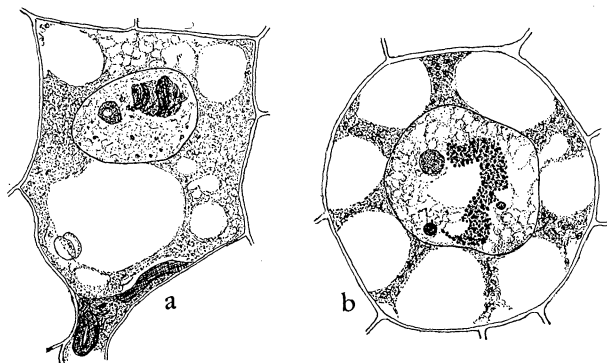


FIG. 18. — Fertilization in *Onoclea sensibilis*.  *a*, sperm as a spiral band within the egg nu-
cleus ; *b*, later stage, the chromatin of the sperm much less condensed and more widely
distributed in the egg nucleus (after Shaw, '98a).

described in the pteridophytes for Onoclea by Shaw ('98 a),
confirmed by Mottier (: 04 a ; : 04 b), and for Adiantum and
Aspidium by Thom ('99).  In these forms the male nucleus
after leaving in the protoplasm of the egg all of the cytoplasmic
structures of the sperm (blepharoplasts, cilia, etc.) enters the
egg nucleus as a more or less spiral body which stains deeply
and is evidently chiefly or wholly chromatic in composition (Fig.
18).  Within the egg the dense structure of the sperm nucleus
becomes looser by the separation of the chromatin granules (Fig.

18, b) but the form of the sperm can be recognized for a long time. The chromatin of the egg nucleus is in a resting condition at this period and the densely packed mass of paternal chromatin is very conspicuous in the loose, delicate network of the female chromatin. The mitosis following fertilization does not occur for several days so that it is not easily studied and the organization of the first cleavage spindle with the history of the maternal and paternal chromosomes has never been followed. But it is clear that we have in the pteridophytes a true fusion nucleus containing for several days both maternal and paternal chromatin within the same nuclear membrane.

There is only one paper that gives any details of fertilization in the bryophytes, a contribution of Kruch ('90) on the liverwort, Riella, which seems to have been generally overlooked in recent literature. After the sperm enters the egg, a male nucleus is organized which increases in size until it is about equal to the egg nucleus. The chromatin in both gamete nuclei is described and figured as forming 8 chromosomes which are organized before the fusion. The two gamete nuclei were observed, but not figured, in contact and it was not possible to distinguish in size the male from the female. This account is then very different from those of the pteridophytes since the sperm nucleus does not enter the egg nucleus but the two fuse side by side and with their chromosomes fully organized. There are, however, some points in Kruch's paper that require more extended investigation and confirmation in the light of modern research.

There is left only the group of the thallophytes where less is known about the detailed behavior of the chromatin during fertilization than in any region of the plant kingdom. The conjugation of the gamete nuclei has been observed in a number of thallophytes, representing all of the higher groups. All of the authors, with the exception of Chmielewski ('90 b) for Spirogyra, describe the product of conjugation as a fusion nucleus, *i. e.*, one in which the nuclear substance of both gametes is contained within a common nuclear membrane. The most detailed accounts of the fusion of gamete nuclei in the thallophytes are those for Fucus (Strasburger, '97 a ; Farmer and Williams, '98).

The sperm of Fucus upon entering the egg loses its cytoplasm and passes rapidly to the egg nucleus as a deeply staining body resembling a plastid in form.  This structure is the male nucleus whose chromatin is so densely crowded that it stains too deeply to show much structure.  Arriving at the side of the female nucleus, about ten minutes after its entrance into the egg, the male nucleus flattens against the female and increases in size so that the chromatin appears less condensed.  The male nucleus is then absorbed so that the paternal chromatin lies within a fusion nucleus but may be distinguished for some time as densely staining material at one side.  A second nucleolus often appears in the fusion nucleus in the vicinity of the paternal chromatin and is probably associated with the entrance of the sperm nucleus, although it is not likely to have been brought in as an organized structure but developed later at the expense of material in the sperm nucleus.  The fusion nucleus remains quiescent for from 20 to 24 hours during which time the paternal chromatin becomes so distributed that it can no longer be followed.  Then two centrospheres with conspicuous radiations appear at opposite poles of the fusion nucleus and the first cleavage spindle is organized.  There is no evidence that either of these centrospheres is brought into the egg by the sperm and both appear *de novo* and independently of one another.

The chief accounts of the fusion of gamete nuclei in thallophytes are as follows:  Closterium and Cosmarium (Klebahn, '91); Rhopalodia (Klebahn, '96); Cocconeis (Karsten, :oo); Sphæroplea (Klebahn, '99; Golenkin, '99); Œdogonium (Klebahn, '92); Coleochæta (Oltmanns, '98); Vaucheria (Oltmanns, '95; Davis, :o4); Fucus (Strasburger, '97 a; Farmer and Williams, '98); Batrachospermum (Schmidle, '99; Osterhout, :oo); Nemalion (Wolfe, :o4); Basidiobolus (Fairchild, '97); Albugo (Wager, '96; Stevens, '99, :o1 b; Davis, :oo); Peronospora (Wager, :oo); Pythium (Miyake, :o1; Trow, :o1); Achlya (Trow, :o4); Araiospora (King, :o3); Sphærotheca (Harper, '95); Pyronema (Harper, :oo).  Most of these papers with others on fertilization in the thallophytes are summarized by Mottier, (:o4 b) in very convenient form for reference.

There is some confusion in the accounts of fertilization in

Spirogyra which should be thoroughly investigated.  Chmielew-
ski ('90 b) in a paper published in Russian and reviewed in the
*Bot. Centralb.*, vol. 50, p. 264, 1892, described a fusion of the
gamete nuclei in the zygospore and an immediate mitosis, with-
out a period of rest, followed at once by a second division of the
daughter nuclei.  These mitoses give the zygospore four nuclei,
two of which unite to form a final resting nucleus in the zygo-
spore while the remaining two fragment and their products
finally break down.  This behavior offers an exception to all
sexual processes so far known in the plant kingdom.  There are
some features which suggest a possible confusion with events as
described in the zygospore of the desmid and the auxospores of
certain diatoms.

The fusion nucleus in the zygospore of Closterium and Cos-
marium (Klebahn, '91) divides into four at the time of germina-
tion and two of these break down while each of the others
becomes the nucleus of the two new desmids that are formed.
There is then in the desmids the division of the fusion nucleus
into four but no secondary nuclear fusions as Chmielewski
reports for Spirogyra.  In certain diatoms, Rhopalodia (Kle-
bahn, '96) and Cocconeis (Karsten, :00), there is a preliminary
division of the nuclei in each of the two cells which form the
auxospore.  In Rhopalodia the mitoses are carried so far that
four daughter nuclei are formed in each diatom and the pro-
toplasm divides into two cells each of which fuses with a
corresponding cell of the companion pair.  In both types the
superfluous nuclei break down so that the conjugating cells have
each a single functional gamete nucleus.  There are then com-
plications in the Conjugales and the diatoms, which make nuclear
studies of the sexual processes exceptionally difficult and we seem
justified in reserving our judgment of the results of Chmielewski
until confirmed.  It seems possible that the mitoses following the
germination of the zygospore in the Conjugales with the attend-
ant nuclear degeneration are reducing divisions in a simple and
primitive type of sporophyte generation but more detailed studies
of nuclear behavior during the formation and germination of the
zygospore will be necessary to settle the discussion.

We have now finished our account of nuclear fusions in the

sexual act (fertilization) but there remains for consideration the behavior of certain cytoplasmic elements introduced into the sexually formed cell, especially chromatophores and the blepharoplast. Since the blepharoplast bears a very close resemblance to the middle piece of the animal spermatozoon, which sometimes becomes a centrosome in the animal egg, a critical comparison of the behavior of these two structures during fertilization is full of interest.

Except for certain accounts of Spirogyra, to be described in the next paragraph, investigators agree that the chromatophores or plastids of gametes never fuse in the sexually formed spore. Plastids have not been found in the sperms of the gymnosperms, pteridophytes, nor bryophytes. The sperms of some algæ also appear quite colorless at maturity but careful examinations have shown in a number of forms a very small chromatophore in the early stages of development. Other less highly differentiated sperms are known to have chromatophores (e. g., Sphæroplea, Cutleria, Volvox). Both gametes in the isogamous types of sexuality among the algæ always have chromatophores or plastids. These have been followed in detail through stages of fertilization in Ectocarpus by Berthold ('81) and Oltmanns ('99), and in Scytosiphon by Kuckuck ('98) where it is evident that they do not unite and there is no reason for believing that different conditions obtain among any of the lower forms such as Ulothrix, Cladophora, Hydrodictyon, etc., although detailed observations are greatly lacking on this point, chiefly because the conjugating cells are generally very small.

Early accounts of the formation of the zygospore of Spirogyra have reported some form of union of the chlorophyll bands of the two gametes. The last work upon the subject, Chmielewski ('90a), reviews the results of previous investigators and gives a detailed account of a species of Rynchonema (Spirogyra). Chmielewski claims that the chromatophore of the gamete (male) that passes over into the other cell (female) becomes disorganized as the zygospore develops. While the chlorophyll band of the female cell retains much of its color, that from the male becomes yellowish and breaks up into fragments which become scattered in the zygospore and finally break down. This inter-

esting account accompanied by clear figures gives an explanation far more in keeping with what we know and might expect of the behavior of chromatophores in resting spores. That the green chromatophore may temporarily become much modified in color and form is well known in some of the red and orange resting spores of the Volvocaceæ and the zygospores of the desmids. In some of these types the form and color of the chromatophores become quite lost for the time being so that studies on their behavior are very difficult. For these reasons it seems probable that the accounts of the fusion of the chromatophore in the zygo-spore of Spirogyra are incorrect. It is very interesting that the gametes of Spirogyra should be so sharply differentiated that the chromatophore of one should be reduced during conjugation in a manner that resembles very closely the behavior of the chro-matophore in highly differentiated sperms.

There is no evidence that the pigment spots, so generally present in the motile gametes of lower forms, ever unite. They have been followed into the zygospore and after the germination of this cell and they remain entirely independent of one another as would be expected from their close relationships to chromato-phores.

The fate of the blepharoplast will now be considered. This structure is especially interesting because of its close analogy to the locomotor apparatus of the animal spermatozoön, which is formed chiefly from one or more centrosomes generally with the coöperation of archoplasm (idiosome, Nebenkern). It is also claimed by a number of zoölogists that in some forms, at least, the centrosomes of the first cleavage spindle are derived from the spermatozoön.

All evidence indicates that the blepharoplast of the plant sperm is left behind in the cytoplasm of the egg when the male nucleus passes into the interior to unite with the female and that centrospheres when present, in the first cleavage spindle, are formed *de novo*. The fate of the blepharoplast is clearly known in Cycas (Ikeno, '98b), Zamia (Webber, : 01) and Ginkgo (Ikeno, : 01). Soon after the large top-shaped sperm of these forms enters the egg, the male nucleus slips out of the spiral blepharoplast, that partially invests it, and, leaving it with

other cytoplasm of the sperm at the end of the egg, passes quickly to the center to unite with the female nucleus. The blepharoplast remains near the periphery of the egg and may be recognized even after the gamete nuclei have united. It finally breaks down and its substance becomes lost in the cytoplasm of the egg. The most complete account of the history of the ble-pharoplast in the fertilized egg is that of Webber ( : 01). We should naturally expect the first cleavage spindle in the cycads and Ginkgo to be developed as in the conifers. Ikeno ( : 01) described clearly an intranuclear spindle in Ginkgo. In the conifers, as previously described, the first cleavage spindle is intranuclear and the fibers are developed freely from a mesh and form a broad poled spindle without centrospheres. So that not only does the blepharoplast break down at a distance from the egg nucleus but we have no reason to think that there is any place for a centrosome in the history of the first cleavage spindle in the gymnosperms.

We do not know clearly the fate of the blepharoplast in the egg of any pteridophyte or bryophyte, although Shaw's ('98a) studies on Onoclea indicate that it breaks down in the cyto-plasm. Our knowledge of the thallophytes is equally incom-plete as regards the history of the blepharoplast in the egg. But both Strasburger ('97a) and Farmer and Williams ('98) have agreed for Fucus that the two centrospheres at the poles of the first cleavage spindle develop *de novo* and independently of one another, and Williams (: 04b) holds the same view for the centrosphere which appears at the side of the fertilized egg of Dictyota. The sperms of the thallophytes are generally very small cells and it may prove a difficult matter to follow their blepharoplasts so that our opinions of events in these forms are likely to be largely inferential from our knowledge in higher groups.

We can safely say that there is no evidence that the blepharo-plast ever enters into the first cleavage spindle which is certainly developed in the spermatophytes and probably in the pterido-phytes without centrosomes or centrospheres. Where centro-somes or centrospheres are known for the first cleavage spindle in the thallophytes (Fucus and Dictyota), the observations indi-

cate that such structures have not come from the blepharoplast. Williams' (: 04b) recent work on Dictyota, while incomplete in the series of stages illustrating the fusion of gamete nuclei (fertilization), presents a very interesting comparison of the development of the first cleavage spindle in fertilized eggs with parthenogenetic eggs. In the fertilized egg there is regularly found a centrosphere which apparently divides into two that separate until they lie at opposite poles of the mature spindle. In the parthenogenetic egg, on the contrary, the spindle is multipolar and develops very irregularly from a kinoplasmic mesh which is intranuclear and there is no sign of centrospheres. Williams believes that fertilization enables the fusion nucleus to form *de novo* a centrosphere external to itself which is not possible for the nucleus of a parthenogenetic egg.

It should be noted that these conclusions are all against the view that the centrosome is a permanent organ of the cell and that the blepharoplast holds any direct relation to centrosomes when present in the first cleavage spindle and inferentially rather strengthens the doubt that the blepharoplast is derived from a centrosome, which point was discussed in our account of the sperm in Section III. However, Ikeno (: 04) in a paper which arrived too late to be treated in Section III, is very positive that blepharoplasts are centrosomes, presenting his evidence clearly, but his explanation of the conditions under which blepharoplasts are formed from the plasma membrane does not seem to me conclusive, especially in the light of Mottier's (: 04a) recent paper on Chara, which also could not be treated in Section III (see *Amer. Nat.*, vol. 38, p. 576, 1904).

### 3. Asexual Cell Unions and Nuclear Fusions.

As stated earlier in the paper, the test of a sexual act must lie with the history of the elements which unite, unless we choose to treat sexuality as a purely physiological process and disregard its relation to morphology in ontogeny and phylogeny. This relation is so precise, *i. e.*, sexuality is so firmly established as a fixed period in the life history of most organisms, that the biologist generally thinks of the sexual process as a part of the

life history, which must take place with as much regularity as
the normal development of any organ.    As a matter of fact,
our knowledge of the structure of sexual elements and the events
of sexual phenomena is almost wholly morphological and for the
present at least it seems safer to treat and define sexuality from
a morphological standpoint.

Under asexual cell unions and nuclear fusions we shall include
a number of interesting phenomena which can be arranged in
three groups : (1) cell fusions which have apparently no sexual
relations ; (2) cell fusions which are substitutes for a normal
ancestral sexual process now suppressed ; and (3) extraordinary
modifications of what may have been originally sexual processes
but which at present serve some peculiar and special function.

In the first group will be included the extensive union of
swarm spores, or the amœboid elements derived from such,
best illustrated in the development of plasmodia ; also such cell
fusions as are clearly for nutritive purposes, as is the union of
the sporophytic portion of the cystocarp of the red algæ with
auxiliary cells and probably also the fusion of sporidia in the
smuts and the conjugation of yeast cells.   The second group
embraces the interesting fusions of the nuclei in teleutospores
of the smuts and rusts and in the basidium with the previous
history of the paired (conjugate) nuclei in the mycelium, perhaps
also the nuclear fusions in the ascus, and such cell unions as
have been reported preliminary to the apogamous development
of the fern sporophyte.   The third group includes the remarka-
ble phenomenon in the embryo sac, the double fusions of the
polar nuclei and the triple fusion of these with the second sperm
nucleus, frequently called "double fertilization."

The well known union of the swarm spores of the Myxomy-
cetes as amœboid cells (myxamœbæ) to form the plasmodium is
one of the best illustrations of a fusion of protoplasm without
sexual significance.   In this general union of hundreds and per-
haps thousands of small cells there are no nuclear fusions so far
as is known, but simply the merging of the cytoplasm to form a
large multinucleate unit.   The whole phenomenon indicates a
coöperative process which is probably economical of nutritive
functions in the semiterrestrial conditions under which plas-

modia live.   It is quite possible that the origin of sex may have
been involved with some of the same principles as those which
bring about the union of swarmers to form a plasmodium, but
the added features of nuclear fusion together with the history
of the sexually formed cells which become in higher groups the
starting point of a sporophyte generation places the sexual act
on a very much higher level of complexity.

There are some records of the union of several zoöspores or
gametes to form a zygospore instead of the usual conjugation in
pairs.   The biciliate gametes of Acetabularia (De Bary and
Strasburger, '77) sometimes conjugate in threes and large
zygotes are figured with five pairs of cilia indicating that as
many gametes entered into their formation.   The gametes of
Protosiphon, described by Rostafinski and Woronin ('77) as in
the life cycle of Botrydium, are reported by them to unite at
times several together and four are so figured.   Klebs ('96, p.
207) in his account of Protosiphon also noted the union of the
gametes in threes especially when in organic solutions.   The
significance of these multiple fusions of swarm spores is not
clear for we know nothing of the nuclear history following the
union.   There is in the habit, however, such a resemblance to
the extensive union of swarmers in the Myxomycetes as to indi-
cate that primarily sexuality may have been concerned chiefly
with cytoplasmic fusions and associated very intimately with
nutritive processes.   I have recently observed several instances
of the conjugation of zoöspores of Saprolegnia when the ele-
ments united in pairs at the ciliated ends and along the sides
exactly as do motile gametes, and the fused cell bore four cilia.
The zoöspores of Saprolegnia are too far removed morphologi-
cally from the highly differentiated sexual organs of the group
to justify the explanation of such conjugation as a sexual act
and we must think of it as due to some peculiarities of nutritive
conditions.

Another class of very interesting cell fusions, associated with
nutritive functions, is presented in the union of the sporophytic
fertile filaments (oöblastema filaments) in the cystocarp of the
Rhodophyceæ with auxiliary cells.   This phenomenon which
was regarded by Schmitz and his followers as sexual in charac-

ter, is considered by Oltmanns ('98b) to have nutritive relations alone. Oltmanns studied the fusion with auxiliary cells in several genera, but especially for Callithamnion and Dudresnaya, and is satisfied that the cell unions concern only the cytoplasm. Fertilization takes place with the fusion of gamete nuclei in the carpogonia and these cells develop the sporophyte generations. The fusion of fertilized carpogonia or filaments derived from them with auxiliary cells, is a feature of a sort of semiparasitic relation that the sporophyte holds to the gametophyte by which it is nourished in part through organic connections with the gametophyte. The nuclei of the sporophytic structures remain quite apart from those of the auxiliary cells so that the union is purely cytoplasmic. This theory of Oltmanns has received strong support through the detailed nuclear studies of Wolfe (: 04) on fertilization and the development of the cystocarp of Nemalion who finds cytological evidence of the sporophytic character of the cystocarp. These papers of Oltmanns and Wolfe have been discussed by myself in the *Bot. Gaz.*, vol. 27, p. 314, 1899, and vol. 39, p. 64, 1905.

Writers have at times attached sexual significance to the conspicuous fusions between sporidia of certain of the Ustilaginales (*e. g.*, Tilletia). But there seems at present no reason to regard this phenomenon as different from the cytoplasmic connections frequently established between cells of hyphæ which are ultimately associated in a common mycelium where the whole forms a close unit with respect to common nutritive relations. Such protoplasmic connections were treated in the first part of this section. Harper ('99a) studied the union of conidia and cells of the promycelium in Ustilago and concluded that the fusions involve the cytoplasm alone, there being no nuclear changes. However, Federley (: 03–: 04 ; review in *Bot. Zeit.*, vol. 62, p. 171, 1904) has observed the migration of a nucleus from one conidium to another in *Ustilago tragopogonis pratensis* (Pers.), and a fusion within the latter. This nuclear fusion was not found in some other forms of Ustilago which behaved as Harper has described. There is nothing in the morphology of the conidia to indicate that they are sexual cells and from what we know of the life history of Basidiomycetes we should look

for sexual processes at other periods more closely associated with the development of teleutospores or basidia.

The conjugation of yeast cells has many points of similarity to the fusion of conidia in the Ustilaginales. This phenomenon has been discovered in an organism obtained from commercial ginger by Barker (:01), which he calls Zygosaccharomyces, and in three species of Schizosaccharomyces by Guilliermond (:03). The conjugation in all forms immediately precedes spore formation and involves a nuclear fusion as well as that of the cytoplasm. The conjugation is followed by division of the fusion nucleus and spore formation in the united cells. The conjugating cells are sisters in the species of Schizosaccharomyces but apparently may not be closely related in Barker's form, Zygosaccharomyces. Both investigators regard the conjugation as a sexual act, and Guilliermond considers the fusion cell to be an ascus with the value of a zygospore. These conclusions do not seem to the writer convincing. Spore formation in the yeasts has not been shown to present any of the peculiarities of nuclear division and free cell formation as described by Harper for the ascus, and until such are established it is hardly safe to conclude that the yeasts are Ascomycetes. Whether or not the conjugation is a sexual process becomes a question of phylogeny and we know too little of the history and relationships of the yeasts to assert that the conjugating cells are morphologically gametes. Again, the view that yeasts are derived from conidia or mycelia of higher fungi which have continued a simple growth by budding in peculiar and favorable media is rather against any view that we are dealing here with a simple or primitive sexual act. There are very striking resemblances to the fusions of conidia in the Ustilaginales, which were described in the previous paragraph and do not appear to be sexual processes. It is unsafe to assume sexuality because the conjugation precedes spore formation, because in most yeasts spore formation takes place regularly without conjugation. Is it not rather another illustration of cell and nuclear fusions related to nutritive processes alone?

Some of the most interesting nuclear fusions, apparently associated with the apogamous development of a sporophyte are

the unions of the pairs of nuclei which enter the cells of the developing teleutospores of the Uredinales and Ustilaginales and the basidium of higher Basidiomycetes.   It has been established through the studies of a number of investigators (chiefly Rosen, '93 ; Dangeard and Sapin-Trouffy, '93 ; Dangeard '93, '94–'95a, c ; Poirault and Raciborski, '95 ; Sapin-Trouffy, '96 ; Maire, : 00 a, b, c, : 02 ; Holden and Harper, : 03) that the æcidiospores and the mycelium derived from them and preceding the development of the uredospores and teleutospores contain pairs of nuclei which divide in such a manner (conjugate division) that the nuclei of the pair are derived through two unbroken lines of succession for a long vegetative period and always maintain complete independence of one another.   Every young teleutospore and basidium contains such a pair of nuclei which shortly fuse so that the mature structure is uninucleate. Dangeard and Sapin-Trouffy have from the first regarded the nuclear fusion within the teleutospore, whether of rust or smut, as a sexual act and the ripe teleutospore a fertilized egg, regardless of the fact that its morphology was not that of any known sexual organs.   Dangeard ('94–'95 c ; : 00) likewise considered the nuclear fusions in the basidium as sexual.   Raciborski ('96) suggested that the series of conjugate mitoses leading to the nuclear fusions in the teleutospore represented a vegetative phase intercalated between the beginning of a sexual act and its finish in the teleutospore.   His explanation, in the light of the recent paper of Blackman (: 04a), was nearest the truth.   Maire (: 02) presents the most extensive account of the nuclear structure in the higher Basidiomycetes previous to and during the formation of the basidia.   He held that the fusion of the paired nuclei (synkaryon) in the basidium was not the whole act of fertilization which must begin with the formation of the paired nuclei.   Maire (: 02, p. 189) gave some suggestions as to how and where the paired nuclei arose but neither he nor any of the authors mentioned above knew clearly their origin.

Blackman (: 04a) has made the most important contribution to the subject of fertilization and alternation of generation in the Uredinales, showing clearly that the paired nuclei appear in the life history of *Phragmidium violaceum* and *Gymnosporangium*

*clavariæforme* just before the development of the æcidium.
They arise in Phragmidium by the migration of a nucleus from
an adjacent cell into an element (the fertile cell) which represents
a female sexual organal. The morphology of the female organ is
not clear but there are suggestions of a structure similar to the
procarps of the Rhodophyceæ and Laboulbeniales. The fertile
cell, after receiving its second nucleus, develops a chain of
æcidiospores, the two nuclei becoming so closely associated in
the paired condition that they divide simultaneously (conjugate
mitosis) from now on until the teleutospores are formed. Thus
the cells of all mycelium beginning with the æcidiospore con-
tain paired nuclei up to the development of the teleutospores,
including of course the uredospores when present. This period
of the life history may be considered as representing a sporophyte
generation, especially since the total of chromatin in the pair of
nuclei is double the amount when the nuclei are solitary. The
sporophyte phase ends with the fusion of the pair of nuclei in
each cell of the teleutospores and in the reduction phenomena
that take place with the germination of the teleutospore, includ-
ing the formation of the promycelium. The sporidia developed
by the promycelium are uninucleate and the cells of the mycelium
derived from them are uninucleate up to the production of the
æcidium. This constitutes the gametophyte phase of the life
history. The spermogonia by their morphology seem to be
male organs, now functionless.

In such of the Uredinales as have no æcidium, as also in the
higher Basidiomycetes and the Ustilaginales, it is probable that
both sexual organs are suppressed since no trace of such struc-
tures has been found. However, we may expect to discover
periods in all of these forms when paired nuclei come into the
life history and after a series of conjugate divisions fuse in the
teleutospore or basidium. Such pairs of nuclei, as stated before,
are known in the Ustilaginales (Dangeard, '93) and in a number
of forms of the Uredinales and the nuclear fusions have been
followed in the teleutospore. Holden and Harper (: 03) have
given an especially clear account of the paired nuclei in the
mycelium and uredospores of Coleosporium together with their
fusion in the teleutospore. Maire (: 02) describes the paired

nuclei (synkaryons) and their fusion in the basidium in a large number of Hymenomycetes and Gasteromycetes.

Evidence is thus accumulating that the cells in the mycelium of higher Basidiomycetes (Hymenomycetes and Gasteromycetes) are binucleate for extended periods previous to the formation of basidia where nuclear fusions always take place. Binucleate cells in the higher Basidiomycetes were first reported by Maire (: 00a ; : 00b), in the tissue preliminary to spore formation. He also confirmed Dangeard ('94–'95c) in his view that only two nuclei unite in the basidium contrary to accounts of Rosen ('93) and Wager ('99, p. 586) which described a succession of fusions involving sometimes as many as six or eight nuclei. Harper (: 02) has given for Hypochnus one of the most complete accounts of the behavior of paired nuclei previous to and during the development of the basidium. The cells of the mycelium of this simple Hymenomycete were found to be binucleate as far back as they were studied which included all of the conspicuous vegetative structure. Only a single pair of nuclei enters the basidium and fuses. Harper's results are then in agreement with the extended observations of Maire (: 02) as are also the detailed studies of Ruhland (: 01) on a number of forms and Bambeke (: 03). Taken together they seem to show clearly that the mycelium, for long periods preliminary to the formation of basidia, contains paired nuclei and that the basidia receive each a single pair, which nuclei fuse. There is thus an exact correspondence between the life histories of the Ustilaginales, Uredinales, and higher Basidiomycetes with respect to the period of paired nuclei and their fusion in the teleutospore or basidium. Dangeard called the fusion in the basidium a sexual act and the structure an oöspore regardless of the morphological difficulties of such a conception. Maire (: 02, p. 202) states that the origin of the paired nuclei is the only phenomenon strictly comparable to fertilization and Blackman's studies support this view. Ruhland (: 01) regards the conditions as a deviation from the normal type of sexuality calling it " intracellular karyogamy." The origin of the paired nuclei is not known for any higher Basidiomycete and the discovery of this period and determination of the events leading to the change from uninucleate mycelium to

binucleate is one of the most interesting problems in this field of botany. This is the point where we should expect to find the remains of sexual organs, if any are present in the higher Basidiomycetes, but it is not likely that they will be found. It seems more probable that the mycelium with the paired nuclei (perhaps sporophytic in character) arises apogamously with a complete suppression of the sexual organs in agreement with such of the Uredinales as have no æcidium and the Ustilaginales.

Blackman's explanation of the history of the paired nuclei in Phragmidium is full of interest. As stated before, he regards the fertile cell which develops a chain of æcidiospores, "as a female reproductive cell which undergoes a process of fertiliza- tion" by a union with an adjacent cell of the mycelium and its reception therefrom of a nucleus. The mycelium then which arises with the æcidiospore is sporophytic in character and so remains until the fusion of the pairs of nuclei in the teleuto- spores. The male organs of the rusts are the spermogonia and the male gametes the spermatia which are of course now func- tionless so that the "process of fertilization" is through the introduction into the female cell of a nucleus which is not phy- logenetically a male sexual element. Blackman's (:04 a, pp. 349–353 ; :04 b) conception of the process as an act of ferti- lization involves some principles which will be briefly outlined.

Blackman believes for Phragmidium "that the primitive normal process of fertilization by means of spermatia has been replaced by fertilization of the female cell through the nucleus of an ordinary vegetative cell" and regards the process as very similar to the phenomenon reported in the apogamous development of ferns by Farmer, Moore, and Digby (:03), which will be consid- ered presently. Blackman points out that normal processes of fertilization such as we have included under the head of "sexual cell unions and nuclear fusions" do not involve in many forms (probably all types with a sporophyte generation) an immediate union of the chromatin of the sexual nuclei which is known to remain distinct during the first cleavage mitosis in a number of types (*e. g.*, Pinus and some other gymnosperms). So there is nothing in the delayed fusion of the paired nuclei up to the teleutospore that is seriously against his explanation of the "fer-

tilization" of the female cell of the Uredinales. Indeed, we
may expect to find that the actual fusion of paternal and mater-
nal chromatin does not take place in the higher plants until the
end of the sporophyte generation in the spore mother cell, as
zoölogists have concluded that such union occurs just previous to
gametogenesis in animals. But is Blackman justified in regard-
ing the phenomenon substituted for the activities of ancestral
sexual organs in Phragmidium, now functionless, as a sexual act
and is it desirable to apply the term fertilization to the phe-
nomenon?

Blackman (:04 b, p. 153) speaks of the introduction of a
nucleus into the fertile cell of the Uredinales and the phenome-
non in the apogamous development of the fern after the account
of Farmer, Moore, and Digby (:03) as "reduced forms of ferti-
lization." It may be questioned whether the use of the term
fertilization is fully justified by the events under discussion. We
are all likely to agree with these authors that the physiological
aspects of the phenomena in the cases under consideration are
similar to sexual acts. But, by the writer, the act of fertiliza-
tion is always considered in phylogenetic relations and strictly
limited to the union of sexually differentiated cells, which are
defined by their morphology through principles of homology.
Whenever one or both of the gametes are suppressed in a life
history and a succeeding generation develops of the sort that
normally follows a sexual act, then such a development is apoga-
mous and the phenomena always introduce features which are
foreign to the processes of normal fertilization and the funda-
mental principles of sexuality.

Perhaps the most important characteristic of sexuality from
an evolutionary standpoint is the fusion of gametes of unrelated
parentage, for in the mingling of diverse protoplasm lie two
factors: (1) a physiological stimulus to development, and (2) an
increased probability of inherited variation which in new combi-
nations will appear to the advantage of the species. Blackman's
"reduced forms of fertilization" which I should prefer to con-
sider apart from normal fertilization as examples of apogamy,
and have so classed in this treatment, do satisfy the physiologi-
cal requirements of a sexual act in that a form of nuclear fusion

is substituted for the union of gamete nuclei but the phylogenetic and evolutionary aspects of sexuality are disregarded. Also, the nuclei that fuse are sometimes very closely related, which is a condition generally avoided in sexual processes except where peculiarities of habit make close inbreeding necessary. It is true that large groups, such as the Basidiomycetes, perhaps certain regions of the Ascomycetes, some Phycomycetes, and some forms of the higher plants and algæ seem to have given up normal sexual processes but there is much evidence that in many cases this loss of sexuality is associated with a certain degree of segregation and with peculiarities of life conditions apart from the normal activities of all organisms or quite different from the ancestral stock. The groups are likely to be distinguished by highly specialized life habits of a sort that make it impossible for inherited sexual organs to function, either through mechanical difficulties or because one or both degenerate. It seems to me much clearer to regard all illustrations of Blackman's "reduced forms of fertilization" under the general term of apogamy even though it may be clear that they are physiological substitutes for sexual acts and to reserve the term fertilization for the union of gametes which can always be clearly identified through morphology in ontogeny and phylogeny. The success of a group even though ancestral sexual processes may be suppressed does not enter into a problem which is at bottom a morphological one. Success is relative and we really have no means of estimating its degree save by actual experiment. It is not likely that any biologist would claim that sexual degeneration is advantageous to any species although the organic world is full of forms which have dispensed with sexuality and still hold their places. These are the reasons why I have grouped cell unions and nuclear fusions as sexual and asexual on a morphological basis founded on phylogenetic principles and why in Section V, we shall devote some attention to the substitutes for sexuality under the head of apogamy.

The Ascomycetes present a phenomenon of nuclear fusion within the ascus which may properly be considered at this time since there is a certain resemblance to the nuclear fusions in the teleutospore and basidium. Dangeard ('94–'95b) gave the

first account of this phenomenon describing it for several forms. The mother cell of an ascus sometimes terminates a hypha but more commonly is situated a little back from the end at a point where the hypha bends abruptly like a knee. The mother cell contains two nuclei, closely related to each other, that unite, after which the fusion nucleus divides to form the ascospores. Dangeard considered this fusion to be a sexual act and the product an oöspore which germinates immediately to form the ascus. He regards the ascus as a sporangium, and equivalent to the promycelium which he calls a conidiophore. Dangeard is not willing to accept any of the evidence that the ascocarp ever results from a sexual act or that sexual organs either functional or abortive are present at any stage in the life history of Ascomycetes. Sexuality, according to him, is reduced to the fusion within the ascus alone. He (Dangeard, '96–'97a, b; : 00) discredits the work of Harper on Sphærotheca, Erysiphe, and Pyronema and the older accounts of De Bary and his pupils on sexual organs of the Ascomycetes. A series of short papers in *Le Botaniste* (: 03, Fas. 1) presents Dangeard's last attack on the work of Harper and a reaffirmation of his peculiar views.

Harper's description of sexual processes in Sphærotheca ('95 ; '96) Erysiphe ('96), and Pyronema (: 00b) are so convincing that, together with our knowledge of sexual organs in the lichens, Laboulbeniales, and Gymnoascales, we must accept the old view of De Bary that the ascocarp represents a development (probably sporophytic) from a sexual phase even though it may be established that there is much apogamy in the Ascomycetes. Harper gives the clearest account of the nuclear fusion in the ascus of any author without, however, committing himself to speculations on its significance. The subject is well summarized in his paper on Pyronema (: 00b, pp. 363, 394). He finds in Erysiphe, Pyronema, and some other forms that the ascus is always developed from a penultimate cell of a hypha which bends sharply so that this cell appears to lie at the tip. There are two nuclei at the end of the ascogenous hypha and these divide simultaneously in a very characteristic manner so that the young ascus receives two of the resultant four nuclei, but each is derived from a different one of the original pair and

consequently they are not sisters. The two nuclei in the ascus then fuse. The origin of the original pair is not known.

No satisfactory explanation of this fusion in the ascus has been advanced. The conditions in the Ascomycetes are not the same as in the Basidiomycetes. There is no series of paired nuclei in the ascogenous hyphæ and no evidence of a delayed fusion of gamete nuclei following a sexual act nor of nuclear fusions associated with the apogamous development of a sporophyte generation. On the contrary, a sexual act with the fusion of gamete nuclei has been clearly established in some forms preliminary to the development of the ascocarp and the nuclear union in the ascus is plainly a supplementary phenomenon. Wager and Harper point out analogies to the account of Chmielewski ('90b) for Spirogyra, considered in a previous part of this section, which described a double nuclear fusion in the zygospore. Thus the primary, sexually formed nucleus of the zygospore is reported to divide into four secondary nuclei, two of which break down while the remaining two unite forming the second and final fusion nucleus of the spore. It is hard to see how these second nuclear fusions can be sexual and Groom ('98) is perhaps correct in considering them superimposed on the sexual act, but their physiological significance is not clear.

Some recent papers support in general Harper's investigations on the ascus. Guilliermond (:04a ; :04b) describes the development of the ascus and ascospores in a number of forms. In an unnamed species of Peziza he found, however, that the ascus developed from the terminal cell of the ascogenous hypha which received two nuclei (that fuse) of the four that are found at the tip. Maire (:03a ; :03b) has reported a similar history for *Galactinia succosa.* Both Maire and Guilliermond note the resemblance of these conditions to the nuclear associations in the young basidium and Maire does not hesitate to consider the two nuclei in the tip of the ascogenous hypha as much reduced synkaryons, (paired nuclei) appearing for a very short period just previous to the nuclear fusions in the ascus. Maire follows Dangeard in denying the sexual processes described by Harper in the Ascomycetes and would allign the events in the ascus with those in the basidium. Guilliermond agrees with

Harper that the number of chromosomes presented in the mitoses within the ascus is large (8, 12, 16, in various species) as against Dangeard and Maire who have claimed that the number is uniformly 4. Guilliermond's account of spore formation in the ascus supports that of Harper (described in Section II) in all essentials and gives especial attention to the structure of the epiplasm and its inclusions.

In summary: the significance of the nuclear fusions in the ascus seems very much of a mystery. If they could be associated with an apogamous development of the ascocarp we should have conditions analogous to those in the Basidiomycetes but following a sexual act as it does in Sphærotheca, Erysiphe, and Pyronema we find a phenomenon whose *raison d' être* is not apparent. However, we do not know the history of the nuclei preceding the group of four at the end of the ascogenous hypha and perhaps it may be discovered that events at this period are concerned with nuclear reduction at the end of a sporophyte generation.

One of the most interesting announcements of recent months is that in a preliminary note of Farmer, Moore, and Digby (: 03) on the nuclear history preceding the apogamous development of a species of Nephrodium. They found that the cells of the prothallus at the point where the sporophyte arose became binucleate by the migration of nuclei from neighboring cells. The two nuclei might remain separate for some time or fuse at once. The authors speak of the whole process "as a kind of irregular fertilization" and Blackman considers it analogous to the entrance of the nucleus into the fertile cell of Phragmidium and the establishment of the paired nuclei in the Uredinales. As we discussed the phenomenon in that connection I considered the use of the term fertilization unfortunate since it included processes which however similar physiologically held no relation morphologically and phylogenetically to normal sexual processes. As stated then, it seems to me much clearer to regard all such apogamous phenomena apart from sexual processes, pointing out as far as possible physiological resemblances but recognizing the wide gap in morphology established by the past evolutionary history of the plant. The interest in the phe-

nomena does not become less by this treatment which certainly avoids much confusion of expression.

There is left for consideration one other group of nuclear fusions which may have sexual significance although such is not obvious, namely the fusions of polar nuclei in the embryo sac of angiosperms and the triple unions of the above with a second sperm nucleus which is often called "double fertilization." Several excellent reviews of this subject have appeared, notably by Strasburger (: oob), Sargant (: oo), Coulter and Chamberlain (: o3), Mottier (: o4a, b), and Guérin (: o4). The explanation of this phenomenon is likely to rest finally upon morphological analysis but at present we are uncertain of the homologies of the polar nuclei and the part they play in the evolutionary history of the endosperm. The most striking theory of the endosperm was proposed by LeMonnier ('87) who suggested that the fusion of the polar nuclei gave origin to a second embryo modified to nourish the normal embryo. One of the polar nuclei is always closely related to the egg nucleus so that in the triple fusions (the sperm with two polar nuclei) we have conditions very close to normal fertilization, the discordant element being not the sperm nucleus but the antipodal polar nucleus. The triple fusions would seem at first thought to be rather favorable to LeMonnier's theory although it is plain that with such a diverse mixture of chromatin from three nuclei the resultant structure can scarcely be called a sporophyte embryo from the very grotesqueness of its make-up. Miss Sargant considers the fusion of the second sperm with the micropylar nucleus as sexual in character but so complicated by the introduction of the antipodal polar nucleus that the result is a bizarre structure not strictly comparable to a normal embryo. In the final solution of this problem we must know whether in phylogeny the sperm and micropylar polar nucleus fused first and the antipodal entered into the process later or whether the polar nuclei began the habit and the second sperm nucleus was drawn afterwards into the activities. Should the first possibility be established the sexual nature of the process would seem clear while in the second the events would be of the nature of asexual nuclear fusions. While we know very little

of the origin and evolution of the endosperm in angiosperms there is some evidence in favor of the second possibility.

Strasburger (:oob) holds that the double and triple nuclear fusions in the embryo sac are not true sexual acts even though they may involve an important principle of fertilization, namely, a stimulus to growth. According to him, sexual processes present two distinct features which he designates as "generative fertilization" and "vegetative fertilization." Generative fertilization deals with the mingling of ancestral hereditary substances in the nuclei and establishes the basis for such characters as hold the species true to its past or introduce new qualities as variations into the germ plasm. Vegetative fertilization brings to the fusion nucleus simply a stimulus to growth such as may be given to unfertilized eggs by changes in their physical and chemical environment. We might apply this classification to many of the examples of asexual nuclear fusions which we have discussed, as in the apogamous development of the fern and the origin of the paired nuclei in the rusts, and they have the elements of vegetative fertilization in Strasburger's sense. But such distinctions are very subtle and it seems rather doubtful whether they add much to the clearness of our conceptions. The growth stimulus of "vegetative fertilization" is always an accompaniment of "generative fertilization" and would be expected of any cell unions or nuclear fusions. The peculiarities of sex lie in the phylogenetic features of the phenomena, *i. e.*, in the union of differentiated gametes with their long evolutionary history and not in the mere fusion of any nuclei at any time.

From this point of view the double fusions of polar nuclei or the triple fusions, when a sperm nucleus becomes involved in the phenomenon, are of very doubtful sexual nature since no phylogenetic connections have been established with the normal sexual processes of the spermatophytes. Indeed, there are many irregularities in the process of endosperm formation which complicate the discussion and make it very difficult to trace relationships. Thus nuclear fusions are described in the late stages of endosperm formation when several of the free nuclei become included in the same cell area by the formation of the cell walls

(Corydalis, Strasburger, '80 ; Tischler, : oo ; Canna, Humphrey, '96).   Such nuclei are known to unite two or more and some-times several together within the cells, forming fusion nuclei with a large and variable number of chromosomes.   In Peperomia and Gunnera the endosperm nucleus results from the fusion of several free nuclei and a number of instances are recorded in which no fusion of the polar nuclei takes place, but the endo-sperm is derived from the division of one or both.   Such irregu-larities, which will probably be greatly increased in number as investigations proceed, indicate that the double and triple fusions preceding the differentiation of the endosperm nucleus are not of phylogenetic importance but are more likely to be special developments in relation to peculiarities of seed forma-tion among the angiosperms rather than of a sexual nature.

However, the triple fusions, when a sperm enters into the composition of the endosperm nucleus, seem to furnish a cytological explanation of the phenomenon of xenia and thus come into very close physiological relations to sexual processes. In xenia we find the effects of hybridization expressed immedi-ately outside of the embryo in the endosperm of the seeds.   If paternal chromatin has entered into the composition of the endo-sperm nucleus or should the sperm nucleus by itself give rise to a series of endosperm nuclei the appearance of paternal char-acters would be expected.   This explanation of xenia was worked out independently by DeVries, Correns, and Webber, the last author having published a particularly clear and full account of the phenomenon (Webber, : oo).   Even though the relation of xenia to hybridization is apparent, it is nevertheless clear that we are dealing with an exceptional process only possible because of the unusual conditions within the embryo sac which allow a second sperm nucleus to enter into the activities of seed forma-tion and it is certainly not established that these activities have any phylogenetic relations to past sexual processes.

Some interesting studies of Nemec (: 02–: 03 ;  : 04) upon asexual nuclear fusions may open the way for explanations of some of the examples which we have considered as asexual in the latter portion of this paper.   Nemec found that mitosis in the root tip of *Pisum sativum* could be checked during anaphase

by treating the material with chloral hydrate so that no walls were formed between the daughter nuclei, which remained in the common mother cell and presently fused with one another. The fusion nucleus presented a double number of chromosomes (twice that of the normal sporophyte) in succeeding mitoses which became reduced in a few hours so that later divisions showed the number characteristic of the sporophyte. Nemec regards nuclear fusions and reduction phenomena as self regulating processes which follow the vital cell fusions characteristic of fertilization. The latter (cell fusions) are then the essential phenomena of sex and nuclear activities follow automatically. Reduction phenomena are atavistic in character. Nemec considers these results in serious conflict with Strasburger's ('94) theory of the periodic reduction of the chromosomes, believing that the number of chromosomes is not so likely to give the characters of the respective sporophyte and gametophyte generations as other factors.

Nemec's contribution is chiefly of interest to us in the present connection as showing that nuclear fusions may result from disturbances of the normal environment very far removed from the conditions that produce sexual cells. And this emphasizes our contention that sexual processes must be judged through phylogenetic analysis and not by physiological resemblances. Thus the nuclear fusions in the ascus, in the basidium, preceding apogamous development of the fern, and perhaps the union of polar nuclei in the embryo sac may be involved with special physiological conditions although they resemble outwardly sexual processes and are sometimes a substitute for these. But nevertheless they are asexual nuclear fusions lacking that fundamental character of sexuality, the result of sexual evolution, namely, a fixed position in a life cycle established by phylogeny and expressed by the classic phrase " ontogeny repeats phylogeny." They are departures from the normal life history either apogamous in character or concerned with some other peculiarity of the plants' existence.

LITERATURE CITED IN SECTION IV, "THE PLANT CELL."

BAMBEKE.
: **03**.  Sur l'evolution nucléaire et la sporulation chez *Hydnangium carneum* Wallr.    Mem. d. l'Acad. Roy. Sci., Litt., Beaux Arts d. Belgique, vol. 54.

BARKER.
: **01**.  A Conjugating Yeast.    Phil. Trans. Roy. Soc. London, vol. 194, p. 467.

BERTHOLD.
'**81**.  Die geschlechtliche Fortpflanzung der eigentlichen Phæosporeen Mitt. a. d. Zoöl. Sta. Neapel, vol. 2, p. 401.

BLACKMAN.
'**98**.  The Cytological Features of Fertilization and related Phenomena in *Pinus sylvestris* L.    Phil. Trans. Roy. Soc.  London, vol. 190, p. 395.

BLACKMAN.
: **04a**.  On the Fertilization, Alternation of Generations and General Cytology of the Uredineæ.    Annals of Bot., vol. 18, p. 323.

BLACKMAN.
: **04b**.  On the Relation of Fertilization to " Apogamy " and " Parthenogenesis."    New Phytologist, vol. 3, p. 149.

CHAMBERLAIN.
'**99**.  Oögenesis in *Pinus laricio*.    Bot. Gazette, vol. 27, p. 268.

CHMIELEWSKI.
'**90a**.  Eine Notiz über das Verhalten der Chlorophyllbänder in den Zygoten der Spirogyren-Arten.    Bot. Zeit., vol. 48, p. 773.

CHMIELEWSKI.
'**90b**.  Materialien zur Morphologie und Physiologie des Sexualprocessen bei den niederen Pflanzen.  (Russian.)  Review, Bot. Centralbl., vol. 50, p. 264.

COKER.
: **03**.  On the Gametophytes and Embryo of Taxodium.  Bot. Gazette, vol. 36, p. 114.

COULTER and CHAMBERLAIN.
: **03**.  Morphology of the Angiosperms.  New York, 1903.

DANGEARD.
'**93**.  La reproduction sexuelle des Ustilaginées.  Compt. Rendus, vol. 117, p. 496.

DANGEARD.
'**94**–'**95a**.  La reproduction sexuelle de *l'Entyloma Glaucii*.  Le Botaniste, vol. 4, p. 12.

DANGEARD.
'94–'95b.  La reproduction sexuelle des Ascomycètes.  Le Botaniste, vol. 4, p. 21.

DANGEARD.
'94–'95c.  Mémoire sur la reproduction sexuelle des Basidiomycètes. Le Botaniste, vol. 4, p. 119.

DANGEARD.
'96–'97a.  La reproduction sexuelle dans le *Sphærotheca Castagnei*.  Le Botaniste, vol. 5, p. 27.

DANGEARD.
'96–'97b.  Second mémoire sur la reproduction sexuelle des Ascomycètes. Le Botaniste, vol. 5, p. 245.

DANGEARD.
: 00.  La reproduction sexuelle des champignons.  Le Botaniste, vol. 7, p. 89.

DANGEARD et SAPIN–TROUFFY.
'93.  Une pseudofécondation chez les Uredinées.  Compt. Rendus, vol. 117, p. 267.

DAVIS.
'96b.  Development of the Cystocarp of *Champia parvula*.  Bot. Gazette, vol. 21, p. 109.

DAVIS.
: 00.  The Fertilization of *Albugo candida*.  Bot. Gazette, vol. 29, p. 297.

DAVIS.
: 01.  The Origin of Sex in Plants.  Pop. Sci. Month., vol. 60, p. 66.

DAVIS.
: 03.  The Evolution of Sex in Plants.  Pop. Sci. Month., vol. 62, p. 300.

DAVIS.
: 04.  Oögenesis in Vaucheria.  Bot. Gazette, vol. 38, p. 81.

DE BARY and STRASBURGER.
'77.  *Acetabularia mediterranea*.  Bot. Zeit., vol. 35, p. 713.

FAIRCHILD.
'97.  Ueber Kerntheilung und Befruchtung bei *Basidiobolus ranarum* Eidam.  Jahrb. f. wiss. Bot., vol. 30, p. 285.

FARMER, MOORE, and DIGBY.
: 03.  On the Cytology of Apogamy and Apospory.  I.  Preliminary Note on Apogamy.  Proc. Roy. Soc. London, vol. 71, p. 453.

FARMER and WILLIAMS.
'98.  Contributions to our Knowledge of the Fucaceæ ; their Life History and Cytology.  Phil. Trans. Roy. Soc. London, vol. 190, p. 623.

FEDERLEY.
: 03–: 04.  Die Copulation der Conidien bei *Ustilago Tragopogonis pratensis* Pers.  Finska Veten. Selsk. Forhandl., vol. 46, no. 2.

FERGUSON.
: 04.	Contributions to the Knowledge of the Life History of Pinus with Special Reference to Sporogenesis, the Development of the Gametophytes and Fertilization. Proc. Wash. Acad. Sci., vol. 6, p. 1.

GARDINER.
'88.	On the Continuity of Protoplasm through the Walls of Vegetable Cells. Arb. d. bot. Inst. Würzb., vol. 3, p. 52.

GARDINER.
' 98.	The Histology of the Cell Wall with Special Reference to the Mode of Connections of the Cells. Proc. Roy. Soc. London, vol. 62, p. 100.

GARDINER.
: 00.	The Genesis and Development of the Wall and Connecting Threads in the Plant Cell. Proc. Roy. Soc. London, vol. 66, p. 186.

GOLENKIN.
'99.	Ueber die Befruchtung bei *Sphæroplea annulina* und über die Structur der Zellkerne bei einigen grünen Algen. Bull. Soc. Imp. Nat. Moscow, 1899, p. 343.

GOROSCHANKIN.
' 83.	Zur Kenntniss der Corpuscula bei den Gymnospermen. Bot. Zeit., vol. 41, p. 825.

GROOM.
'98.	On the Fusion of Nuclei among Plants. Trans. and Proc. Bot. Soc. Edinburgh, 1898.

GUÉRIN.
: 04.	Les connaissances actuelles sur la fécondation chez les phanérogams. Paris, 1904.

GUILLIERMOND.
: 03.	Recherches cytologiques sur les levure. Rev. Gen. d. Bot., vol. 15, pp. 49, 104, 166.

GUILLIERMOND.
: 04a.	Contribution à l'étude de la formation des asques et de l'epiplasme des Ascomycètes. Rev. Gen. d. Bot., vol. 16, p. 49.

GUILLIERMOND.
: 04b.	Recherches sur la karyokinèse chez les Ascomycètes. Rev. Gen. d. Bot., vol. 16, p. 129.

HARPER.
'95.	Die Entwickelung des Perithecium bei *Sphærotheca Castagnei.* Ber. deutsch. bot. Gesellsch., vol. 13, p. 475.

HARPER.
'96.	Ueber das Verhalten der Kerne bei der Fruchtentwickelung einiger Ascomyceten. Jahrb. f. wiss. Bot., vol. 29, p. 655.

HARPER.
'99a.	Nuclear Phenomena in certain Stages of Development of the Smuts. Trans. Wisc. Acad. Sci., Arts, Lett., vol. 12, p. 475.

HARPER.
:02. Binucleate Cells in certain Hymenomycetes. Bot. Gazette, vol.
    33, p. 1.

HARPER.
:00. Sexual Reproduction in *Pyronema confluens* and the Morphology
    of the Ascocarp. Annals of Bot., vol. 14, p. 321.

HAUPTFLEISCH.
'88. Zellmembran und Hüllgallerte der Desmidiaceen. Mitt. a. d.
    naturwiss. Ver. f. Neu-Vor. u. Rügen, 1888.

HAUPTFLEISCH.
'95. Die Ortsbewegung der Bacillariaceen. Mitt. a. d. naturwiss. Ver.
    f. Neu-Vor. u. Rügen, vol. 27.

HILL.
:01. The Histology of the Sieve-tubes of Pinus. Annals of Bot., vol.
    15, p. 575.

HOLDEN and HARPER.
:03. Nuclear Division and Nuclear Fusion in *Coleosporium sonchi-
    arvensis* Lev. Trans. Wisc. Acad. Sci., Arts, Lett., vol. 14, p. 63.

HUMPHREY.
'96. The Development of the Seed in Scitamineæ. Annals of Bot.,
    vol. 10, p. 1.

IKENO.
'98b. Untersuchungen über die Entwickelung der Geschlechtsorgane
    und den Vorgang der Befruchtung bei *Cycas revoluta.* Jahrb. f.
    wiss. Bot., vol. 32, p. 557.

IKENO.
:01. Contribution a l'étude de la fécondation chez le *Ginkgo biloba.*
    Ann. Sci. Nat., Bot., ser. 8, vol. 13, p. 305.

IKENO.
:04. Blepharoplasten im Pflanzenreich. Biol. Centralbl., vol. 24, p.
    211.

KARSTEN.
:00. Die Auxosporenbildung der Gattungen Cocconeis, Surirella, und
    Cymatopleura. Flora, vol. 87, p. 253.

KIENITZ–GERLOFF.
'91. Die Protoplasmaverbindungen zwischen benachbarten Gewebsele-
    menten in der Pflanze. Bot. Zeit., vol. 49, pp. 17, 33, 49, 65.

KIENITZ–GERLOFF.
:02. Neue Studien über Plasmodesmen. Ber. deutsch. bot. Gesellsch.,
    vol. 20, p. 93.

KING.
:03. Observations on the Cytology of *Araiospora pulchra* Thaxter.
    Proc. Boston Soc. Nat. Hist., vol. 31, p. 211.

KLEBAHN.
'91. Studien über Zygoten. I. Die Keimung von Closterium und
    Cosmarium. Jahrb. f. wiss. Bot., vol. 22, p. 415.

KLEBAHN.
  '92.  Studien über Zygoten. II. Die Befruchtung von *Œdogonium Boscii.* Jahrb. f. wiss. Bot., vol. 24, p. 235.
KLEBAHN.
  '96.  Beiträge zur Kenntniss der Auxosporenbildung. I. *Rhopalodia gibba* (Ehrenb.) O. Müller. Jahrb. f. wiss. Bot., vol. 29, p. 595.
KLEBAHN.
  '99.  Die Befruchtung von *Sphæroplea annulina* Ag. Festsch. f. Schwendener, p. 81, 1899.
KLEBS.
  '96.  Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen. Jena, 1896.
KNY.
  :04.  Studien über intercellularen Protoplasma. Ber. deutsch. bot. Gesellsch., vol. 22, pp. 29, 347.
KOENICKE.
  :01.  Ueber Ortsveränderungen von Kernen. Sitz. niederrh. Gesellsch. f. Nat. u. Heilk. z. Bonn, 1901, p. 14.
KOENICKE.
  :04.  Der heutige Stand der pflanzlichen Zellforchung. Ber. deut. bot. Gesellsch., vol. 21, p. 66.
KOHL.
  '91.  Protoplasmaverbindungen bei Algen. Ber. deutsch. bot. Gesellsch., vol. 9, p. 9.
KOHL.
  '97.  Die Protoplasmaverbindungen der Spaltöffnungsschliesszellen und der Moosblattzellen. Bot. Centralbl., vol. 72, p. 257.
KOHL.
  :00.  Dimorphism der Plasmaverbindungen. Ber. deutsch. bot. Gesellsch., vol. 18, p. 364.
KOHL.
  :02.  Beiträge zur Kenntniss der Plasmaverbindungen in den Pflanzen. Beiheft z. Bot. Centralbl., vol. 12, p. 343.
KRUCH.
  '90.  Appunti sullo sviluppo degli organi sessuali e sulla fecondazione della *Riella clausonis* Let. Malpighia, vol. 4, p. 403.
KUCKUCK.
  '98.  Ueber die Paarung von Schwarmsporen bei Scytosiphon. Ber. deutsch. bot. Gesellsch., vol. 16, p. 35.
KUHLA.
  :00.  Die Plasmaverbindungen bei *Viscum album.* Bot. Zeit., vol. 58, p. 29.
LAND.
  :02.  A Morphological Study of Thuja. Bot. Gazette, vol. 34, p. 249.

LAWSON.
:04a.   The Gametophytes, Archegonia, Fertilization and Embryo of
        *Sequoia sempervirens.*   Annals of Bot., vol. 18, p. 1.

LAWSON.
:04b.   The Gametophytes, Fertilization and Embryo of *Cryptomeria
        japonica.*   Annals of Bot., vol. 18, p. 417.

LeMONNIER.
'87.    Sur la valeur morphologique de l'albumen chez les angiospermes.
        Journ. d. Bot., vol. 1, p. 140.

MAIRE.
:00a.   Sur la cytologie des Hymenomycètes.   Compt. Rendus, vol. 131,
        p. 121.

MAIRE.
:00b.   Sur la cytologie des Gasteromycètes.   Compt. Rendus, vol. 131,
        p. 1246.

MAIRE.
:00c.   L'évolution nucléaire chez les Endophyllum.   Journ. d. Bot.,
        vol. 14, p. 80.

MAIRE.
:02.    Recherches cytologiques et taxonomiques sur les Basidiomycètes.
        Bull. Soc. Mycol. d. France, vol. 18.

MAIRE.
:03a.   Recherches cytologiques sur le *Galactinia succosa.*   Compt.
        Rendus de l'Acad. Sci. Paris, Nov. 9, 1903.

MAIRE.
:03b.   La formation des asques chez les Pezizes et l'évolution nucléaire
        des Ascomycètes.   Compt. Rendus d. Séan. d. l. Soc. Biol., vol.
        55, p. 1401.

MEYER.
'96.    Die Plasmaverbindungen und die Membranen von *Volvox globator*,
        *aureus* und *tertius* mit Rucksicht auf die tierischen Zellen.   Bot.
        Zeit., vol. 54, p. 187.

MEYER.
:02.    Die Plasmaverbindungen und die Fusionen der Pilze der Flori-
        deenreihe.   Bot. Zeit., vol. 60, p. 139.

MICHNIEWICZ.
:04.    Ueber Plasmodesmen in den Kotyledonen von Lupinus-Arten und
        ihre Beziehung zum interzellularen Plasma.   Oester. bot. Zeitsch.,
        vol. 54, p. 165.

MIEHE.
:01.    Ueber die Wanderungen des pflanzlichen Zellkernes.   Flora, vol.
        88, p. 105.

MIYAKE.
:01.    The Fertilization of *Pythium deBaryanum.*   Annals of Bot., vol.
        15, p. 653.

MIYAKE.
: **03a.**  On the Development of the Sexual Organs in *Picea excelsa.*
Annals of Bot., vol. 17, p. 351.
MIYAKE.
: **03b.**  Contribution to the Fertilization and Embryogeny of *Abies bal-
samea.*  Beiheft z. Bot. Centralbl., vol. 14, p. 134.
MOENKHAUS.
: **04.**  The Development of the Hybrids between *Fundulus heteroclitus*
and *Menedia notata* with especial Reference to the Behavior of
the Maternal and Paternal Chromosomes.  Amer. Journ. of Anat.,
vol. 3, p. 29.
MONTGOMERY.
: **01.**  A Study of the Germ Cells of Metazoa.  Trans. Amer. Phil. Soc.,
vol. 20, p. 154.
MOTTIER.
'98.  Ueber das Verhalten der Kerne bei der Entwickelung des Embryo-
sacks und die Vorgänge bei der Befruchtung.  Jahrb. f. wiss. Bot.,
vol. 31, p. 125.
MOTTIER.
: **04a.**  The Development of the Spermatozoid in Chara.  Annals of Bot.,
vol. 18, p. 245.
MOTTIER.
: **04b.**  Fecundation in Plants.  Carnegie Inst. Washington, 1904.
MÜLLER.
'98–'99.  Kammern und Poren in der Zellwand der Bacillariaceen.  I,
II.  Ber. deutsch. bot. Gesellsch., vol. 16, p. 386 ; vol. 17, p. 432.
MURRILL.
: **00.**  The Development of the Archegonium and Fertilization in the
Hemlock Spruce (*Tsuga Canadensis* Carr.)  Annals of Bot., vol.
14, p. 583.
NEMEC.
: **02–:03.**  Ueber ungeschlechtliche Kernverschmeltzungen.  I, II, III.
Sitz. kön. böhm. Gesellsch. Wiss., 1902–03.
NEMEC.
: **04.**  Ueber die Einwirkung des Chloralhydrats auf die Kern- und
Zelltheilung.  Jahrb. f. wiss. Bot., vol. 39, p. 645.
OLTMANNS.
'95.  Ueber die Entwickelung der Sexualorgane bei Vaucheria.  Flora,
vol. 80, p. 388.
OLTMANNS.
'98a.  Die Entwickelung der Sexualorgane bei *Coleochæte pulvinata.*
Flora, vol. 85, p. 1.
OLTMANNS.
'98b.  Zur Entwickelungsgeschichte der Florideen.  Bot. Zeit., vol. 56,
p. 99.

OLTMANNS.
   '99.   Ueber der Sexualität der Ectocarpeen.   Flora, vol. 86, p. 86.
OSTERHOUT.
   : 00.   Befruchtung bei Batrachospermum.   Flora, vol. 87, p. 109.
POIRAULT and RAČIBORSKI.
   '95.   Sur le noyau des Urédinées.   Journ. d. Bot., vol. 9, p. 318.
RAČIBORSKI.
   '96.   Ueber den Einfluss äusserer Bedingungen auf die Wachstumsweise
          des *Basidiobolus ranarum*.   Flora, vol. 82, p. 107.
REINHARDT.
   '92.   Das Wachstum der Pilzhyphen.   Jahrb. f. wiss. Bot., vol. 23,
          p. 479.
ROBERTSON.
   : 04.   Studies in the Morphology of *Torreya californica* Torrey. II.
          The Sexual Organs and Fertilization.   New Phytologist, vol. 3,
          p. 205.
ROSEN.
   '93.   Studien über die Kerne und die Membranbildung bei Myxomyceten
          und Pilzen.   Cohn's Beitr. z. Biol. d. Pflan., vol. 6, p. 237.
ROSTAFINSKI und WORONIN.
   '77.   Ueber *Botrydium granulatum*.   Bot. Zeit., vol. 35, p. 649.
RUHLAND.
   : 01.   Zur Kenntniss der intracellularen Karyogamie bei den Basidio-
          myceten.   Bot. Zeit., vol. 59, p. 187.
RUSSOW.
   '83.   Ueber den Zusammenhang der Protoplasmakörper benachbarter
          Zellen.   Sitz. dorpater naturf. Gesellsch., 1883.
SAPPIN–TROUFFY.
   '96.   Recherches histologiques sur la famille des Urédinées.   Le Botan-
          iste, vol. 5, p. 59.
SARGANT.
   : 00.   Recent Work on the Results of Fertilization in Angiosperms.
          Annals of Bot., vol. 14, p. 689.
SCHMIDLE.
   '99.   Einiges über die Befruchtung, Keimung und Haarinsertion von
          Batrachospermum.   Bot. Zeit., vol. 57, p. 125.
SCHRAMMEN.
   : 02.   Ueber die Einwirkung von Temperaturen auf die Zellen des Vege-
          tationspunktes des Sprosses von *Vicia foba*.   Verhand. d. natur-
          hist. Vereins d. preuss. Rheinlande, vol. 59.
SCHUTT.
   '99.   Centrifugales Dickenwachstum der Membran und extramembran-
          oses Plasma.   Jahrb. f. wiss. Bot., vol. 33, p. 594.

SCHUTT.
: **00a.** Die Erklärung des centrifugalen Dickenwachstum der Membran. Bot. Zeit., vol. 59, p. 245.

SCHUTT.
: **00b.** Centrifugale und simultane Membranverdickungen. Jahrb. f. wiss. Bot., vol. 35, p. 470.

SHAW.
**'98a.** The Fertilization of Onoclea. Annals of Bot., vol. 12, p. 261.

SMITH.
: **00.** The Haustoria of the Erysipheæ. Bot. Gazette, vol. 29, p. 153.

SMITH, ISABEL.
: **04.** The Nutrition of the Egg in Zamia. Bot. Gazette, vol. 37, p. 346.

STEVENS.
**'99.** The Compound Oösphere of *Albugo bliti.* Bot. Gazette, vol. 28, p. 149.

STEVENS.
: **01b.** Gametogenesis and Fertilization in Albugo. Bot. Gazette, vol. 32, p. 77.

STRASBURGER.
**'80.** Zellbildung und Zelltheilung. Jena, 1880.

STRASBURGER.
**'82.** Ueber den Bau und Wachsthum der Zellhaüte. Jena, 1882.

STRASBURGER.
**'94.** The Periodic Reduction of the Number of Chromosomes in the Life History of Living Organisms. Annals of Bot., vol. 8, p. 281.

STRASBURGER.
**'97a.** Kerntheilung und Befruchtung bei Fucus. Jahrb. f. wiss. Bot., vol. 30, p. 351.

STRASBURGER.
: **00b.** Einige Bemerkungen zur Frage nach der doppleten Befruchtung bei den Angiospermen. Bot. Zeit., vol. 58, p. 293.

STRASBURGER.
: **01.** Ueber Plasmaverbindungen pflanzlichen Zellen. Jahrb. f. wiss. Bot., vol. 36, p. 493

SUTTON.
: **02.** On the Morphology of the Chromosome Group in *Brachystola magna.* Biol. Bull., vol. 4, p. 24.

SUTTON.
: **03.** The Chromosomes in Heredity. Biol. Bull., vol. 4, p. 231.

TANGL.
**'79–'81.** Ueber offene Communicationen zwischen den Zellen des Endosperms einiger Samen. Jahrb. f. wiss. Bot., vol. 12, p. 170.

TERNETZ.
: **00.** Protoplasmabewegung und Fruchtkörperbildung bei *Ascophanus carneus.* Jahrb. f. wiss. Bot., vol. 35, p. 273.

THAXTER.

'96.   Contribution towards a Monograph of the Laboulbeniaceæ.   Mem.
       Amer. Acad. Arts and Sci., vol. 12.

THOM.

'99.   The Process of Fertilization in Aspidium and Adiantum.   Trans.
       Acad. Sci. St. Louis, vol. 9, p. 285.

TISCHLER.

: 00.  Untersuchungen über die Entwickelung des Endosperm und der
       Samenschale von *Corydalis cava.*   Verh. naturhist.-med. Ver.
       Heidelberg, vol. 6, p. 351.

TOWNSEND.

'97.   Der Einfluss des Zellkerns auf die Bildung der Zellhaut.   Jahrb.
       f. wiss. Bot., vol. 30, p. 484.

TROW.

: 01.  Biology and Cytology of *Pythium ultimum* n. sp.   Annals of Bot.,
       vol. 15, p. 269.

TROW.

: 04.  On Fertilization in the Saprolegnieæ.   Annals of Bot., vol. 18, p.
       541.

WAGER.

'96.   On the Structure and Reproduction of *Cystopus candidus* Lev.
       Annals of Bot., vol. 10, p. 295.

WAGER.

'99.   The Sexuality of Fungi.   Annals of Bot., vol. 8, p. 575.

WAGER.

: 00.  On the Fertilization of *Peronospora parasitica.*   Annals of Bot..
       vol. 14, p. 263.

WEBBER.

: 00.  Xenia, or the Immediate Effect of Pollen in Maize.   Bull. 22, Div.
       Veg. Path. and Phys., U. S. Dept. of Agric.

WEBBER.

: 01.  Spermatogenesis and Fecundation of Zamia.   Bull. 2, Bureau
       Plant Ind., U. S. Dept. of Agric.

WILLIAMS.

: 04b. Studies in the Dictyotaceæ.   II.   The Cytology of the Gameto-
       phyte Generation.   Annals of Bot., vol. 18, p. 183.

WILSON.

: 00.  The Cell in Development and Inheritance.   New York, 1900.

WOLFE.

: 04.  Cytological Studies on Nemalion.   Annals of Bot., vol. 18, p. 607.

WOYCICKI.

'99.   On Fertilization in the Coniferæ.   (Russian.)   Review, Bot. Zeit.,
       vol. 58, p. 39, 1900.